REPETITIVE HEAD TRAUMA IN EARLY COGNITIVE AND BEHAVIORAL DECLINE AND FRONTOLOIMBIC DYSFUNCTION.

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Human Movement Science Curriculum in the Department of Allied Health Sciences in the School of Medicine.

Chapel Hill
2017

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ABSTRACT

Michael David Clark: Repetitive Head Trauma in Early Cognitive and Behavioral Decline and Frontolimbic Dysfunction.
(Under the direction of Kevin Guskiewicz)

Converging evidence suggests an association between exposure to recurrent concussion and detriments to cognitive and behavioral health later in life. Many believe the clinical symptoms present in those with a history of recurrent trauma may be related to underlying onset or progression of neurodegeneration, though the relationship remains incompletely understood. Chronic traumatic encephalopathy (CTE), a neurodegenerative disease characterized by an accumulation of hyperphosphorylated tau deposits, has been posited as the disease most likely in individuals with clinical symptoms who have been exposed to repetitive head trauma. This thesis focuses on the phenotype of early cognitive and behavioral decline in former professional football players with a history of recurrent concussions and high-volume exposure to subconcussive impacts. This population is believed to be at an especially high risk of developing CTE. We identify 18 cases of mild cognitive and behavioral impairments in this highly exposed group and compare them to 15 healthy controls – former professional football players with a similar playing background.

Using a battery of neuropsychological tests and psychiatric symptom surveys, the impairments in this group are characterized. The neural underpinnings of such impairments are examined through diffusion-weighted and functional magnetic resonance imaging (MRI) with a focus on the frontolimbic system. This network, composed of both prefrontal and limbic system regions is thought to be involved in CTE-related pathology, giving rise to the posited clinical syndrome associated with neurodegeneration in exposed individuals. Our results corroborate similar studies in this area by showing marked behavioral dysfunction in the cognitively impaired

group coinciding with a loss of white matter integrity in the bilateral uncinate fasciculi. The functional consequences of such neuroanatomical changes are evidenced by enhanced interference of emotionally valent distractors on working memory task performance and corresponding over-activation of the bilateral temporal poles when such distractors are present.

This work furthers our understanding of the neural correlates to mild clinical impairments in a group at high-risk for developing neurodegeneration. Such information provides critical insight into pathophysiology and may contribute to early diagnosis, provide targets for pharmacological or psychotherapy, and improve prognosis of future decline.

To my wife, Stephanie.

ACKNOWLEDGMENTS

Many favors have been paid to me throughout the course of this project and I would like to express my sincere appreciation to everyone who helped me along the way. This work would not be possible without the efforts of many people to whom I am deeply indebted.

I am very grateful to the participants in this project. Many of them traveled from across the country to engage in research that they believe will benefit the broader population of football players, knowing that they will not reap the benefits of this work themselves. Seeing the impact of neurodegenerative disease on their lives and the lives of their loved ones was a poignant reminder of the importance and urgency of this area of research. In many cases, participants went out of their way to refer other people to the study and promote our work in former player community. Their patience and selflessness cannot be understated.

I would like to thank Amy Matthews for her efforts in recruiting participants and assisting with the numerous chores that arise in launching a clinical research study. Her experience and dedication to the project in the face of the many logistical complications we encountered was admirable and I'm extremely grateful for her help. I'd also like to thank Shannon Leith for her efforts both in recruitment and in administering the neuropsychological testing battery. Both of these women are much beloved by the study participants, and myself, for their warm approach and clear and effective communication.

I'd like to thank Leah Cox Thomas for her help in mining historical records to build the registry from which these participants were drawn and for the many, many times she fielded questions from me regarding past studies. I would never have got off the ground without Leah's remarkable competence and efficiency.

I would like to thank Dr. Cecile Ladouceur at the University of Pittsburgh for providing an E-Prime script for the fMRI paradigm used in the study. I'd also like to thank Chris Foster for his assistance in modifying this script to fit our specific protocol. Thank you to Dr. Martin Styner, who provided guidance on the diffusion-weighted imaging analysis and helped troubleshoot problems along the way.

I would like to recognize the funding agencies that made this work possible: The National Institute of Neurological Disorders and Stroke (F30-NS090816), the North Carolina Translational and Clinical Research Institute (#550KR121512), the Matthew Gfeller Foundation Trust, and the University of North Carolina Medical Scientist Program (NIH T32 GM008719).

Lastly, I'd like to acknowledge the immeasurable contribution of the late Dr. Eugene Orringer to my professional growth and development. Always a fierce student advocate and champion, Dr. O went out of his way to provide support, wisdom, and guidance to me in the first half of my medical and graduate training.

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fMRI	Functional magnetic resonance imaging
CTE	Chronic traumatic encephalopathy
MCI	Mild cognitive impairment
NFL	National Football League
DWI	Diffusion weighted imaging
DTI	Diffusion tensor imaging
AD	Alzheimer's disease
NPI-Q	Neuropsychiatric inventory – questionnaire
mTICS	Modified telephone interview of cognitive status
CDR	Clinical dementia rating
FA	Fractional anisotropy

CHAPTER 1: INTRODUCTION

Overview and Background

This project concerns the association between large-volume exposure to concussive and subconcussive head impacts and late-life, neurological health of former professional football players. The overarching goal of this project is to identify and characterize early cognitive and behavioral impairments in former professional football players and to better understand the neural underpinning of such impairments. In this thesis, I introduce these clinical problems and discuss their public health significance. In doing so, relevant observational and epidemiological evidence are presented along with current gaps in our understanding of the relationship between head trauma and neurological health. This background will provide context to the project. The specific aims will then be presented with the underlying hypotheses that motivate their focus. Finally, this chapter concludes with an abbreviated discussion of core concepts integral to the project; this discussion is furthered in the review of literature constituting Chapter Two.

While relatively few studies have examined the long-term effects of recurrent, sport-related concussion, several trends have emerged, including: increased risk of depression,[1] cognitive impairment,[2,3] Alzheimer's disease (AD) and other causes of dementia,[4] and neurodegenerative cause of death.[5] Despite these observations, little is known about the long-term neuroanatomical and neurophysiological changes associated with recurrent concussions and the phenotype of early cognitive decline in a population with exposure to such repetitive head trauma.

Postmortem brain histopathology studies of former contact athletes and former military veterans, populations with well-described exposure to head trauma, have revealed a consistent pattern of neurodegeneration referred to as chronic traumatic encephalopathy (CTE).[6,7] The clinical presentation of CTE is believed to consist of behavioral disturbances, primarily depression, aggression and agitation, anxiety, and cognitive impairments, such as memory problems and executive dysfunction.[8] Additionally, Parkinsonism has also been noted as feature of the disease.[6,9,10] In light of these findings, and despite some overlap of symptoms, CTE is believed to be neuropathologically and clinically distinct from other forms of dementia, particularly AD.[11-14] However, in vivo evidence of CTE is limited and there is contention concerning its status as a distinct clinical syndrome as no diagnostic criteria have been established. Consequently, there is an absence of an evidence-based framework for diagnosis and prognosis of CTE. Nevertheless, striking neuropathological studies of CTE make urgent the need to better characterize CTE in vivo.

This study examines a group at high risk of CTE, former professional football players aged 56 to 76 with nine or more years of football experience and a history of recurrent concussions. The aims and hypotheses of this project comprise cognitive and neuropsychiatric symptomatology and diffusion-weighted and functional imaging of the frontolimbic neural network. The predictions of these hypotheses will be tested independently, though the three aims of the project are intimately related; the neural networks studied in Aims 2 and 3 are believed to be the neural substrate of the symptoms assessed in Aim 1. If our hypotheses are supported by the results of the project, we will have gained critical information about early impairments in those with a history of recurrent concussive and subconcussive head impacts. This may elucidate targets for interventions meant to arrest the underlying disease process or it may provide useful information for the diagnosis and prognosis of CTE.

On the other hand, if we fail to support our hypotheses, we will still derive useful information from this work. Failing to reject the null hypotheses may suggest that we cannot detect subtle deficits in the early stages of disease using the methodology within this study. The variability between subjects may be too great to discern smaller effect sizes between the groups in our modest sample size. In either case, we will have generated a rich and complex dataset that will allow us to examine the relationships between multiple imaging modalities, measures of postural control, and a thorough complement of neuropsychological data.

The base population from which we will draw our clinical sample is former professional football players with at least three years of experience at the professional level and who report experiencing at least three concussions in their lifetime. Cases must have a clinical dementia rating (CDR) of 0.5, suggestive of mild cognitive impairment, while controls must be free of cognitive impairments. By holding head impact exposure constant, we will be able to discern neuropsychiatric and neurophysiological differences between the former athletes who appear to be developing underlying neurodegenerative disease and those who are not. Using a focused battery of neuropsychological tests and psychiatric symptom surveys, we will further characterize the cognitive and behavioral function of the groups. Using diffusion-weighted and functional imaging, we will probe the underlying neural substrate of these domains of function.

Based on previous CTE literature and observational studies of former athletes with recurrent concussion, we hypothesize that in comparison to non-athletes, the former professional football athletes will have increased behavioral and psychiatric symptomatology related to aggression, depression, and irritability that is mediated by frontolimbic network dysfunction. This dysfunction of the frontolimbic system will be evidenced by reduced white matter integrity in the cingulate bundle, uncinate fasciculi, and forceps minor, and greater activation of frontolimbic regions in response to the working memory-emotional face distractor fMRI task.

Specific Aims and Hypotheses

Aim 1: To specify differences in cognitive and neuropsychiatric function between former athletes with and without cognitive impairments.

Hypothesis: Previous investigations on athletes with recurrent concussion converge on marked limbic dysfunction as a feature of decline unique to those with repetitive head trauma. We predict that the former professional football players with mild cognitive impairment (MCI) will have greater behavioral and psychiatric symptomatology than former NFL players without MCI. Specifically, we expect the MCI group to have greater symptoms related to anxiety, depression, and impulsive aggression.

Aim 2: To specify differences in white matter integrity of major white matter tracts within the frontolimbic network between former athletes with and without cognitive impairments.

Hypothesis: Trauma caused by recurrent concussion reduces the white matter integrity of association fibers within the frontolimbic neural network. Previous studies have shown a reduction in integrity within the uncinate fasciculus and cingulate bundle in those with a history of recurrent concussion compared to healthy controls. Accordingly, we expect the impaired group will have reduced fractional anisotropy within these tracts. We also expect mean diffusivity and orientation dispersion index of these tracts will be increased with a corresponding decrease in the intracellular volume.

Aim 3: To specify differences in functional neural recruitment in response to a working memory, emotional faces distractor N-back task between former athletes with and without cognitive impairments.

Hypothesis: Given the involvement of the frontolimbic system in the CTE literature, we expect this network will be dysfunctional in the impaired group. The task paradigm used in this study is designed to engage both the frontoparietal and frontolimbic networks in order to successfully complete the task. We expect that the impaired group will have

worse performance on the N-back task when facial distractors are present, and that they will show an increased signal in task irrelevant regions (dedifferentiation) when the cognitive load of the task is maximal.

Clinical Application and Limitations

Mounting evidence suggests recurrent concussions are associated with CTE neurodegeneration. However, specific criteria of a clinical syndrome associated with underlying CTE have not been elucidated, in large part due to a lack of in vivo studies. Informant interview studies of autopsy confirmed CTE in former professional football players suggest a clinical syndrome consisting of multi-domain cognitive impairments and behavioral disturbances. Interestingly, literature concerning late life cognition in those with a history of moderate or severe TBI suggest these injuries are risk factors for Alzheimer's disease (AD) and that those with AD and a prior history of moderate or severe TBI are more likely to have higher scores on the Neuropsychiatric Inventory – Questionnaire (NPIQ).[15] Specifically, they are more likely to report depression, aggression, and irritability and they have a lower odds ratio of reporting issues with memory, the most commonly and severely affected cognitive domain in typical AD. This may be taken as evidence that these patients were possibly misdiagnosed; erroneous diagnoses of AD have been reported previously for cases of confirmed CTE.[6] Regardless of its clinical label, the pattern of cognitive impairments in those with a history of repetitive head trauma may not follow the typical progression of AD in which episodic memory is the prominent cognitive impairment.

The focus of this project is on the early disease process, when cognition is only mildly impaired. This is a critical window in which changes in cognition and behavior become noticeable and clinically observable, but they have not become extensive enough to impact independent daily living. During this time, if the underlying process were arrested or slowed, it is

possible that patients would be able to retain independence for a longer period of time. Identifying early cognitive and behavioral changes in those with a history of concussive and subconcussive impact exposure may identify those who are most likely to go on to have progressive neurodegeneration.

Beyond differences in cognitive functioning, we believe there will be important differences in neuropsychiatric status between our impaired and asymptomatic subjects, and that these symptoms will be related to changes in the frontolimbic network caused by repetitive stress to the neurons composing the network. Thus, the current model of MCI as a clinically observable prodrome for a broad range of distinct neurodegenerative diseases is non-specific and poorly constructed for non-AD related decline. Behavioral domains are not currently covered by the criteria of MCI, which reduces the clinical utility of such a label in the former NFL player population. The recent construct of mild behavioral impairment (MBI), may distinguish typical AD from other types of neurodegeneration (namely, frontotemporal lobar degeneration), and may be more appropriate in the context of CTE. In such cases, cognitive function may not be the primary manifestation, but rather behavioral impairments. This aligns with the postulation of Stern et al., who suggests two variants of CTE, one of which is more behaviorally dominant while the other presents with largely cognitive impairments.[8,14] By studying those with a high exposure to head impacts (both concussive and subconcussive), we can begin to appreciate the unique symptoms and characteristics of early decline in those believed to be at highest risk of progressing to a clinical syndrome corresponding to an underlying CTE neuropathology.

It is unknown if repetitive head trauma is sufficient for the development of this phenotype of cognitive decline, or if such trauma only modifies the expression of an underlying neurodegenerative process through damage to specific neural networks. This question of causality is not addressed in the current study design. Instead, the focus of this work is centered on exactly how early cognitive decline is expressed in those with a known and substantial

history of concussions and subconcussive impact exposure.

Furthermore, this work does not address the underlying neuropathology of CTE. Considerable strides have been made in characterizing the patterns of tau neurofibrillary tangle (NFT) deposition in those with autopsy-confirmed CTE. The neuropathological consensus criteria are adequately specific to distinguish CTE from other forms to neurodegeneration, though there is overlap between the diseases, and the presence of one does not exclude the presence of the others. In a subset of our study sample, we have augmented our imaging protocol with positron emission tomography with the ligand [^{18}F]-THK-5351, which binds to intracellular tau aggregates. This may provide complementary evidence for the presence of CTE pathology in the early stages of cognitive decline. These data are outside the scope of this project as funding limits our ability to get a PET scan for all subjects. In the absence of PET imaging, we will be unable to determine the tau deposition burden in our subjects.

A further limitation of this work is the characterization of head trauma in the former athlete cohort. By necessity, we must rely on self-report for the number and clinical course (i.e. presence of loss of consciousness or post-traumatic amnesia and duration and extent of symptoms) of concussion episodes and the length and character (e.g. playing position, playing time, practice frequency, etc.) of the former athletes' football careers. We know concussions can occur through a variety of biomechanical mechanisms; this is true even if we limit our focus to sport-related concussions. It is to be expected that a concussion occurring through a frontal impact will have a substantially different distribution of forces over the cerebrum than a concussive impact to the back, top, or side of the head. Complicating the matter further, concussions occur not only through linear accelerations, but angular accelerations as well. Lastly, the clinical outcome following concussion can vary widely and may be related to the extent of neurological insult and the course of recovery. Some former players report losing consciousness due to a blow to the head and returning to the game without rest, leaving them

open to another insult that may again go unresolved. Quantifying the clinical course following each reported concussion is subject to recall bias and poor recognition of concussion symptoms in the past.

Because of the variety of concussion mechanisms and our inability to access information about the specific mechanism and clinical course for each self-reported concussion, we will only be able to discern the common elements of concussions. In other words, the specific character of exposure to concussive and subconcussive impacts may add significant heterogeneity to the imaging results. However, the frontolimbic regions and the white matter tracts connecting them are ventral, caudal, and near midline, making this network susceptible to shear forces in a variety of concussive mechanisms. Thus, we expect the heterogeneity of imaging findings to be minimal within the network of interest. This hypothesis predicts the symptom profile with respect to neuropsychiatric functioning will also be common in the group with impairments, though it is acknowledged this assumption may not be correct.

The scope of this work is intended to be broad but is incomplete. The aforementioned small sample size issues and delimitations, necessary for feasibility and timeliness, make the contribution of this work modest when viewed in isolation. Further research along this line of investigation will require a prospective design, or at least an age-stratified, cross-sectional design, that examines subjects in various stages of decline. Furthermore, future work should take into account the varying levels of traumatic exposure; not only number of concussive episodes, but also, if possible, the nature of each episode (mechanism, clinical course, and treatments). In this work, we simply cannot reliably examine these multitudinous factors. Thus, the goal of this work is to provide a foundation of preliminary data on which a study of greater scope can be envisioned.

CHAPTER 2: LITERATURE REVIEW

Sport-related Concussions

An estimated 1.6 to 3.8 million sport-related traumatic brain injuries (TBIs) occur in the United States annually,[16] with an estimated 10 million all-cause TBIs occurring globally each year.[17] Concussions, or mild TBIs, are the most common form, accounting for an estimated 80% of all severities of TBI. At least 20% of emergency department visits for concussions with loss of consciousness are attributed to sports and recreational activities.[18] Organized team sports account for approximately half of emergency department visits for concussion in the 14-18 year old age range.[19]

Concussion is a concern at every competitive level in contact sports.[20-23] Of particular concern is American football, which has one of the highest incidences of sport-related concussion across all levels of competition.[24-26] More than 7 million U.S. high school students compete in interscholastic sports every year[27] with over one million high school athletes playing football.[28] Furthermore, over 250,000 children ages 5-14 participate in Pop Warner football every year.

In addition to athletics, concussions are a concern for military service-members as well; as many as 20% of Iraq War veterans suffered a mild TBI while deployed.[29] TBI has been described as the “signature injury” of the Iraq and Afghanistan Wars, partly due to the emphasis on explosive devices in modern combat. Blast mechanisms of concussive injury are fundamentally a different problem than those encountered in sport-related TBI, however, these account for only a portion of the total TBIs in the military population. While the breadth of the problem is largely unknown in this unique population, there is a major focus on the cumulative

effects of multiple concussions, as will be discussed in the next subsection.

While great strides have been made in recognizing concussion symptoms and promoting proper management, as many as 50% of sport-related concussions are believed to be unreported.[30] The factors contributing to the underreporting of concussive injury are multifactorial and include gaps in knowledge concerning concussion symptoms, beliefs that the injury is not serious, and unwillingness to be removed from competition.[30,31] A study of confidential symptom reporting by concussed collegiate athletes found that they were more forthcoming in reporting symptoms when it would not be considered in return-to-play decisions.[32] These lines of converging evidence underscore the unwillingness of athletes to report their injuries and their symptoms, particularly when it affects their ability to continue playing their sport, and thus, determining the true incidence of concussive injury is difficult. This is likely especially true in the military population, though it is a fundamentally difficult problem to assess.

Recurrent Concussions

Athletes with a history of concussion are at increased risk of additional concussions in the future.[33-35] In the National Football League, 29.4% of athletes had a repeat concussion in the period of 1996-2007 and 51% of concussions during this period were repeat concussions. This rate did not decrease in the period from 2002-2007, despite more conservative management practices.[36] In a 2007 study of 2,552 former NFL players, over 60% reported one or more concussions in their playing career with 24% reporting *three or more* concussions.[1] As more information is disseminated through the lay press about the signs and symptoms of concussion, the self-reported number of concussions in this population is expected to increase as more players become aware that they likely failed to recognize concussions they experienced.

Recurrent concussions are a major concern as the cumulative effects of the injury are

largely unknown. Athletes tend to report a greater number of symptoms after repeat injury [37] and a history of multiple previous concussions is associated with prolonged recovery. [38] High school athletes with three or more concussions tend to develop more acute symptoms upon subsequent injury. [39] These findings suggest that there is a cumulative effect of recurrent concussions in the acute setting. These findings have been a focus in the military population as well, with increased concussive symptoms[40] and sleep disturbances [41] being associated with a history of repeated mild TBI.

Long-term effects of recurrent concussions in former professional football players have been shown to include increased risk of depression and cognitive impairment (**figure 2.1**[1,2]). Athletes reporting three or more concussions had five times the prevalence of clinically-diagnosed mild cognitive impairment (MCI) and three times the prevalence of significant memory problems, compared to players reporting no concussions.[2] Furthermore, in the former NFL player population, there is evidence of increased prevalence of mild cognitive impairment, [42] dementia from any cause,[4] and possibly earlier onset Alzheimer's disease.[2] The overall age-adjusted prevalence ratio for AD was 1.37 (95% CI: 0.98 — 1.56), indicating the football retirees tended to have higher prevalence than other U.S. males of the same age.[2] Some have argued, however, that AD may be a misdiagnosis of those who actually have neuropathological evidence of CTE. In addition, the 9-year risk of depression increases with more self-reported concussions (risk ratio of 2.2 – 5.8 depending on number of concussions reported) [43] and athletes with three or more concussions have a three times greater prevalence of depression than those without history of concussion.[1]

Given the mounting evidence of persistent deficits following recurrent concussion, it is believed that such recurrent mild TBIs are clinically more similar to moderate TBI in terms of outcomes. While a single concussive episode may not lead to lasting morbidity, the cumulative effects of recurrent injury are noticeable and are increasingly being studied.

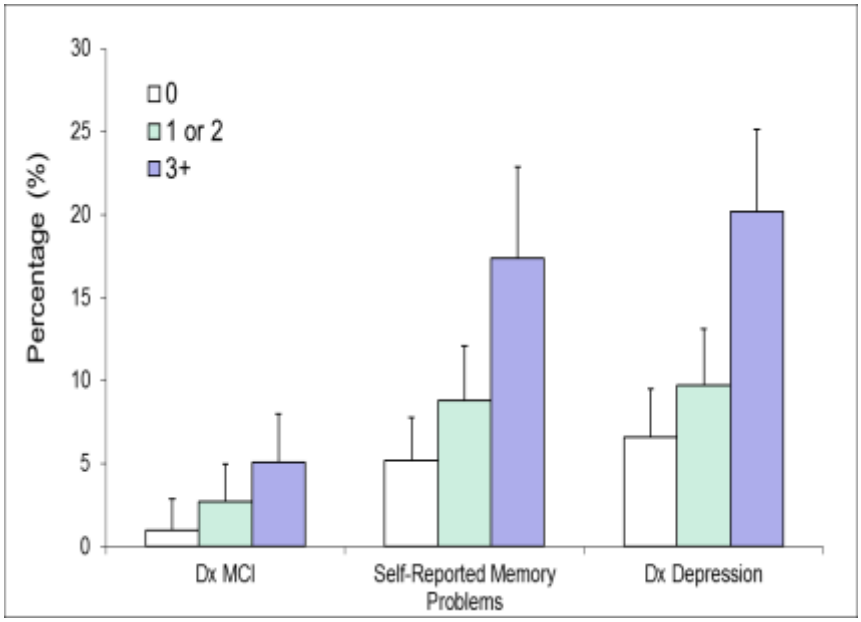


Figure 2.1: Cognitive complaints of retired NFL players with history of concussion. (Adapted from Guskiewicz et al. 2005, 2007; refs 1, 2).

Neurodegeneration

While a single moderate or severe traumatic brain injury (TBI) is a strong risk factor for developing Alzheimer's disease (AD), [44] the connection between a single concussion and late-life cognition is less clear. In the case of recurrent concussions, however, there is mounting evidence to suggest the injuries have a cumulative and lasting effect. Chronic traumatic encephalopathy (CTE) has been posited as a pathologically and clinically distinct neurodegenerative disease secondary to repetitive head trauma.

The mechanism underlying the link between recurrent concussion and the development of cognitive and psychiatric symptoms later in life is understudied. An association between repetitive head trauma and Chronic Traumatic Encephalopathy (CTE) has been proposed.[6,8,10,45] CTE is described as a neurodegenerative disease, distinguishable from Alzheimer's disease and other forms of dementia on the basis of post-mortem neuropathology. Histological studies on the brains of deceased contact athletes and military veterans have shown a distinct pattern of hyper-phosphorylated tau deposition.[11-13,45,46]

There is controversy regarding CTE's classification as a distinct neuropathology secondary to head trauma.[3,47-49] Thus far, in vivo evidence of CTE is lacking, and no clear clinical syndrome has been identified and studied prospectively. The lack of diagnostic criteria is a major criticism of the classification of CTE as a distinct neurological disease. Furthermore, there is contention over the neuropathological findings associated with the CTE phenotype. Tau and amyloid deposition has been observed in several neurodegenerative disease states,[50] and has even been observed in subjects who had no previous history of cognitive or behavioral impairments.[51,52]

In 2015, The National Institutes of Health convened a consensus conference to define neuropathological criteria for the diagnosis of CTE.[53] This consensus laid out the pathognomonic findings, supporting criteria for diagnosis, and exclusionary findings in

diagnosing CTE. The primary feature was noted to be "*abnormal perivascular accumulation of tau in neurons, astrocytes, and cell processes in an irregular pattern at the depths of the cortical sulci*." Spatially, it was noted that tau distribution was variable, but that hippocampal and neocortical involvement were common. However, involvement in CA1 of the hippocampus, in association with co-localized amyloid plaques, was more likely to be found in Alzheimer's disease. Furthermore, the depth of tau deposition within the cytoarchitecture of the cortex is variable between AD and CTE. NFTs in CTE tend to be found in the deeper layers, whereas in AD, the deposits are more superficial.[54]

The clinical presentation of CTE is poorly understood. There have been attempts to describe the phenotype related to confirmed cases of CTE. Interviews with family members and other close informants of deceased athletes with confirmed CTE post mortem reveal a syndrome comprising memory problems, executive dysfunction, behavioral and personality changes, depression, and aggression.[8] Preliminary results of a recent studies demonstrate the presence of tau deposits in symptomatic former football players distributed in a pattern consistent with previous autopsy studies.[55-57] Specifically, in these studies, tau ligand signal was localized to frontolimbic regions, which underlies the clinical expression of behavioral disturbance. Several lines of investigation provide converging evidence that the nature of neurodegenerative disease is unique in those with a history of concussive and subconcussive exposure. Thus, it is likely that the expressions of early, prodromal stages of the neurodegenerative disease affecting those with a history of TBI would be different from those without such history.

Aim 1: Neuropsychiatric Symptomatology

A major focus of the neuropsychiatric domain of cognitive function in this study will be on impulsive aggression, anxiety, and depression. These symptoms are often present in those with confirmed CTE on autopsy through post-mortem informant interviews.[8,9,58] While the interviews are likely biased by recall, especially in the case where the cause of death was a suicide, there remains a concern over the function of the frontolimbic neural network in those with a history of head trauma. In this study, these symptoms of frontolimbic dysfunction will be defined dimensionally rather than categorically. The symptomatology in the subjects may not warrant a clinical label of, say, generalized anxiety disorder or unipolar depression (or whatever labels correspond to the categorical classification system one is using), but it is affecting their lives enough to report it on a survey or in an interview. Rather than detecting the presence or absence of a categorical label, we will seek to quantify the three chosen dimensions of emotion, while using categorical labels for their overall cognitive and behavioral function. In the MCI population as a whole (i.e. not restricting to MCI-AD), the presence of one or more of apathy, aggression, irritability, anxiety, or depressive symptoms is approximately 50% prevalent.[59] However, much of the previous literature has used a categorical approach, as in report of the symptoms or not, as reflected herein.

The selection of the specific symptoms of anxiety, depression, and aggression is based on the literature concerning neuropsychiatric outcomes following TBI. Many of these studies examine single episodes of TBI ranging the spectrum from mild to severe. Outcomes following repetitive trauma are far less studied and prone to strong selection biases. It is hypothesized that the outcomes of repetitive concussions/mild TBI are more similar to the moderate or severe TBI forms. However, where concussion and mild TBI literature is available, it is overviewed here.

The symptomatology of anxiety and depression following TBI is becoming an increasing

studied phenomenon.[60,61] Previous concussion has been shown to be a strong risk factor for depression in adolescents.[62] The incidence rate of depression after TBI is between 15.3 to 33% and the prevalence ranges of 18.5 to 61%.[63] These wide variations reflect considerable heterogeneity in definitions, length of follow-up, and diagnostic criteria and instruments used in the studies included in the systematic review. Concussed athletes are more likely to report depressive symptoms following their injury, even after being returned to play.[64] The relationship between TBI and depression is evident not only in the acute setting post-injury, but also years later. In former NFL players, the 9-year risk of depression increased with more self-reported concussions (risk ratio of 2.2 -- 5.8 depending on number of concussions reported)[43] and athletes with three or more concussions have a three times greater prevalence of depression than those without history of concussion.[1] Anxiety symptoms are rarely studied in the absence of depression as these are so often comorbid. However, one meta-analysis of 41 studies in adult, non-penetrating TBI observed 11% were diagnosed with generalized anxiety disorder (GAD) and 37% reported clinically significant levels of anxiety.[65] The rates of GAD increased with injury severity (11% for mild and 15% for severe) and the diagnoses most prevalent 2-5 years after injury.

The construct of aggression is complex and heterogeneous; the variant under study is that of an impulsive, violent response to a perceived provocation, particularly with anger or hostile affect.[66] This is often called impulsive aggression, to distinguish it from the more premeditated, goal-oriented form associated with borderline or antisocial personality disorders. Here we will consider the term “aggression” to mean the impulsive variant. Aggression is commonly observed following TBI with as many as 25% of those with moderate to severe injury being classified as aggressive following injury.[67] Depending on definitions and study methodologies, the rates of aggression after TBI range from 23 – 79%.[68] Aggression and associated behaviors are often the most difficult to treat following brain injury.[69] There is much

less research specific to mild TBI and the development of aggression.

Our preliminary data collected in the Brain and Body Health Program for NFL retirees suggest neuropsychiatric symptoms, as assessed by the NPIQ are highly prevalent in this population (**Table 2.1**). Data from 55 participants in the program with two or more self-reported, lifetime concussions show elevated distress scores for both the retirees and caregivers. This population is a mix of those with cognitive impairments and those who are cognitively normal; we expect the prevalence is greater in those retirees with MCI. The connection between these domains of neuropsychiatric functioning cannot be ignored. The frontolimbic neural network has been associated with each of these symptoms, as will be discussed in the succeeding subsections.

To assess neuropsychiatric symptomatology in this group, we will use a focused set of easily implement clinical measures. While we expect that mean symptom scores will be elevated in the MCI group, it may be the case where just a subset of those with MCI express neuropsychiatric symptoms. Accordingly, we will conduct a secondary analysis in which we define subjects as being the in "exposed, mild behavioral impairments" (MBI) group using the Neuropsychiatric Inventory Questionnaire (NPIQ). The NPIQ was originally developed and widely used for assessment of psychopathology in dementia. It is now used to investigate neuropsychiatric manifestations of other brain disorders and has demonstrated sensitivity to changes in neuropsychiatric status following a traumatic brain injury. The NPIQ evaluates 12 neuropsychiatric disturbances (apathy, irritability, euphoria, disinhibition, delusions, hallucinations, agitation, dysphoria, anxiety, aberrant motor behavior, night-time behavior disturbances, and appetite and eating abnormalities). The NPIQ is administered to an informant of the patient, typically a spouse, relative, or caregiver. The NPIQ, however, has not been validated in subjects with remote history of head trauma, and thus, it may not be sensitive to detecting the clinical neuropsychiatric presentation of expression. The various definitions of

“mild behavioral impairment” differ in their clinical criteria and method of assessment. Our definition is limited to the cut-offs described in the methodology in Chapter 3. To reduce analytical bias, the analyses presented herein are using the a priori cut-offs. Future directions would include secondary analyses across finer sub-stratifications based on specific psychiatric symptoms (e.g. irritability, apathy, depression).

Table 2.1: Symptoms reported by informants of NFL retirees with 2+ previous concussions

NPIQ Symptom Category	Frequency	Severity (1-3)	Caregiver Distress (0-3)
Agitation/Aggression	54.50%	1.97	2.07
Depression/Dysphoria	63.60%	2.00	1.79
Apathy/Indifference	59.30%	1.97	1.55
Irritability/Lability	75.90%	2.03	1.96

*Unpublished data; Center for the Study of Retired Athletes; N=55

Depression symptoms were measured using the Beck Depression Inventory (BDI), a 21-question multiple-choice, self-report inventory and one of the most widely used instruments for measuring the severity of depression. Depression is the most commonly associated neuropsychiatric symptom with aggression at all time-points post-TBI.[67] We use the total inventory score to assess depressive symptoms. A suicide action plan is in place if the subject reports thoughts of suicidality. The patient health questionnaire 9-item scale (PHQ-9) was used as a supplement to the BDI as this instrument is rapidly administered and asks questions that are complementary.

Aggression was assessed with the Buss-Perry Aggressiveness Questionnaire (BPAQ). The BPAQ has been found to be valid across a multitude of populations to evaluate aggressiveness. The long form version has a high score of 60 with 29 total questions. It is capable of breaking aggressive behavior into four subscales, physical aggression (nine items), verbal aggression (five items), anger (seven items), and hostility (eight items). The BPAQ is given as a seven point-Likert scale ranging from “extremely uncharacteristic” to “extremely characteristic.”

Anxiety symptoms were assessed using the Generalized Anxiety Disorder 7-item scale (GAD-7),[70] which has been validated in multiple populations in its ability to detect generalized anxiety, panic, social anxiety, and post-traumatic stress disorder (PTSD). This behavioral measure assesses the subject's anxiety levels over the past two weeks using seven questions. Answers range from "not at all" to "nearly every day" with total scores ranging from 0-21. The accepted cut-point for generalized anxiety is a score of 10. Alternate cut-points are 0-4 (minimal), 5-9 (mild), 10-14 (moderate), and 15-21 (severe).

Suicidality has appeared in the CTE literature as a possible feature of the disease process.[46] This is not, however, accepted as a defining characteristic of the disease because of a lack of supporting evidence outside of anecdotal cases. Much of the associations drawn

between CTE and suicide are fallacious of the form "p*ost hoc*, *ergo propter hoc* (after TBI, therefore because of TBI) or *cum hoc*, *ergo propter hoc (*with TBI, therefore because of TBI)." [71] Suicidality was not noted to be part of the phenotype by McKee et al. in their review of all CTE cases up to 2009. [6] Despite the fallacious treatment of suicidality and suicidal ideation as part of the clinical syndrome, these problems remain of grave concern in those status post TBI. There is evidence to suggest TBI is a risk factor for later life suicide and that, alarmingly, there is no time period within 15 years post-injury in which the suicides tend to occur; some suicides occurred over 25 years post injury.[72]

Severe TBI appears to have a greater risk ratio than mild TBI, though this is clouded by the vague definitions common in mild TBI literature. It is often that such injuries are attributed to non-injury related factors, not the injury per se, and that this attribution to the injury is more common in severe TBI.[73] In this same publication, Oquedo et al. noted that those with mild TBI were more likely to be aggressive than non-mild TBI subjects, that they were more likely to be suicide attempters, and that suicide attempts were most predicted by aggression and hostility, not mild TBI status. They concluded that suicide and mild TBI share antecedent risk factors of aggression and hostility, though this was a cross-sectional design with retrospective recall of psychiatric traits, and thus, strictly delimited in its causal inference. Depression remains the single greatest risk factor for suicide[74] and there is considerable overlap between suicide risk factors and post-TBI sequelae, including depression.

The Frontolimbic Neural Network

The frontolimbic network is composed of both limbic and frontal regions of interest. Specifically, the analyses in this project will focus on the dorsolateral prefrontal cortex (DLPFC), ventrolateral prefrontal cortex (VLPFC), orbitofrontal cortex (OFC), amygdala, hippocampi, cingulate gyri, and insula. The white matter connections of interest include the cingulum (both

the cingulate gyrus ending and the angular bundle) and uncinate fasciculus. This network and regions comprising the frontolimbic network have been shown to be dysfunctional after concussive injury,[60,75,76] particularly in patients who go on to have symptoms of anxiety. The frontolimbic network has yet to be explored in a population with recurrent concussion and long duration exposure to head impacts.

The regions composing the frontolimbic network are associated with a wide variety of emotional and cognitive functions. The amygdala plays a central role in the experience and generation of negative emotions.[77-79] The balance of connectivity between the frontal and limbic regions is critical for regulating response to emotional stimuli.[80] The associated regions in the frontal cortex appear to inhibit or suppression an emotional reaction to stimuli.[81] The activity of the amygdala and ventromedial prefrontal cortex are inversely correlated during regulation of negative emotions.[82] In addition to mediating a psychological response to emotion, the network has a close physiological connection to the hypothalamic-pituitary-adrenal axis.[82,83] Young adults with a family history of depression showed lower activation of the dorsolateral prefrontal cortex in response to the Hariri task involving presentation of emotional faces (see succeeding section for more information on this task).[84]

Countless studies have been conducted on the regions that are comprised by the frontolimbic network, particularly in relation to emotional processing, cognitive function, and TBI; very few examine the interaction of these three factors. Mayberg 1999, proposed the limbic – cortical network connections were critical in mediating symptoms of major depression.[85] In fact, this hypothesis has spawned several clinical trials examining the efficacy of transcranial magnetic or deep-brain stimulation as a treatment for depression.[86] The orbitofrontal cortex has been identified as a key region in the expression of anxiety, spanning several distinct subtypes of anxiety disorders.[87,88] Lastly, aggression and violence are believed to be caused by dysfunction of the frontolimbic network, specifically through a loss of inhibitory control from the

prefrontal cortex.[66]

Two key studies have direct relevance to the proposed project; one was conducted in the population of interest, former athletes with history of concussion, the other in those with typical MCI leading to AD. Goswami et al. 2015, in a hypothesis-naïve machine learning study, observed that axial diffusivity of the uncinate fasciculus and thickness of the OFC negatively correlated with scores of aggression in athletes with a history of concussion.[89] In Trzepacz et al. 2013, volume and thickness of several regions within the frontolimbic network (amygdala, anterior cingulate, orbitofrontal, among others) were shown to correlate with NPI-Q scores of agitation and aggression in those with early Alzheimer's disease.[90]

The extent to which the frontolimbic network is involved in the early cognitive changes associated with a CTE-like syndrome is the focus of this project. A preponderance of evidence suggests the emotional functioning of those affected by CTE may be compromised. This leads to the hypothesis that the underlying neural substrate of such function is likely affected by the neuropathological processes in a differential manner. We hypothesize that frontolimbic dysfunction, caused by the accumulation of NFTs, likely mediates the neuropsychiatric symptoms observed post-TBI and in the former athlete cohort with history of concussion and football exposure. Specifically, the loss of inhibitory control from the prefrontal cortex on limbic system structures may lead to greater expression of aggression, depression, and anxiety. Corroborating this hypothesis, histopathological studies of CTE have shown dense NFTs in limbic structures.[6] Furthermore, several investigations converge on frontolimbic circuit dysfunction following traumatic brain injury as a mediator of post-traumatic depression and anxiety.[60,75,76,91-93] Using advanced neuroimaging, we can test this hypothesis in vivo. Specifically, we use a multi-modal approach, incorporating complementary information from diffusion-weighted and functional MRI. Diffusion-weighted imaging will give valuable insight into the structures composing the frontolimbic system and how white matter may be affected. Using

a task-based fMRI paradigm, we can assess the functional communication between frontal and limbic regions. The specific task employed will be described in the succeeding section. The involvement of the frontolimbic network in early cognitive decline due to CTE may be a target for the development of tailored therapeutic drugs. Furthermore, the extent of disease involvement in the frontolimbic network may be a marker for the progression of CTE.

Neuroimaging

Neural network dysfunction is a hallmark of TBI; this is true even for mild TBI in which there is no focal damage to neurological structures apparent on cranial tomography or MRI.[94] The confluence of shear, tensile, and compressive forces on neuronal cell bodies and their axons causes microscopic, diffuse axonal injury and acute impairment of synchronous neuronal firing.[95] Thus, the nature of network dysfunction is apparent not only structurally, but functionally as well. Following acute injury, the extent and timeline of recovery for the neural networks affected by the injury are poorly understood. Some evidence suggests persistent changes to neurophysiology and neuroanatomy may be noticeable years after injury. Acute damage to microtubules from the acute injury results in hyper-phosphorylation of tau with subsequent aggregation within the neurons. Such damage may serve as a nidus for continued deposition of NFTs, becoming sufficiently extensive to impact neuronal function. Some posit that these NFTs may even be passed trans-synaptically to other neurons within the network.[94]

Because the nature of network dysfunction in TBI is believed to be structural, functional, and pathological, a multi-modal approach to neuroimaging is appropriate to best elucidate the neural mechanisms of CTE neurodegeneration. Accordingly, we will collect a variety of neuroimaging sequences, focusing on magnetic resonance imaging. Specifically, we will acquire diffusion-weighted imaging, resting state and task-related functional MRI, and structural MRI sequences (Magnetization Prepared Rapid Gradient Echo, MPRAGE). For a subset of subjects,

as funds allow, we will also simultaneously collect PET data using a ligand specific to tau NFTs, [^{18}F]THK-5351. In the next subsections, I will provide a justification for the inclusion of such modalities, with emphasis on diffusion tensor imaging (DTI) and functional MRI data collected by our research group. As the complexity of the data is such that analysis is likely to extend well beyond my tenure with the Matthew Gfeller Center, the focus of aims 2 and 3 is on the hypothesis of the frontolimbic network dysfunction.

Aim 2: Diffusion-Weighted Imaging

Diffusion-weighted imaging exploits the magnetic properties of water diffusion and the differential diffusion properties of neurological tissues. In bundled, well-organized white matter, diffusion is more ordered and predictable; more net diffusion occurs along the length of axons compared to along the transverse plane of the axon. When damage occurs to white matter, the diffusion-weighted signal is often altered. In TBI, damage to white matter can affect the connections between cortical and subcortical regions in a differential manner depending on the mechanism of the injury. However, there are consistencies in the literature regarding the location of damage in groups with variable mechanisms of injury. Specifically, the corpus callosum has been identified as the most commonly reported major white matter tract affected by TBI.[96] The fornix, uncinate fasciculus, cingulum bundle, and hippocampus have all been observed to have lower integrity measures after TBI using DTI.[97]

In the former football player population, many studies using DTI have been published. Former players with mood or cognitive symptoms were distinguishable either from control subjects,[98,89] or asymptomatic players[99] using DTI. Goswami et al. found that mean and radial diffusivity in the uncinate fasciculus could be used as the basis for classifying athletes with a history of concussions from controls. Additionally, FA in the white-matter skeleton has been shown to be significantly associated with symptoms of depression[99] and impulsivity.[89]

Interestingly, the forceps minor was correlated to depression scores.[99] In their asymptomatic collegiate athletes, Tremblay et al. did not observe significant clinical correlates to FA, however, they did observe significant relationships between a test of visual memory and other diffusivity metrics, including axial, radial, and mean diffusivity.[100]

Three previous studies have found no differences in fractional anisotropy (FA) when comparing retired NFL players with a history of concussions to age-matched, non-athlete controls. Hart et al. 2013,[98] reported no white matter FA differences between asymptomatic players (i.e. no deficits in cognition or mood) and controls (n=12 in each group). Strain et al. 2013,[99] found no differences in FA between non-depressed NFL players with significant concussion history (n=21) and matched controls (n=22). In Goswami et al. 2015,[89] the authors restricted their TBSS analysis of white matter FA to the uncinate fasciculus and found no differences between former NFL players (n=19) and controls (n=17). Delving further into the white matter structure of these former athletes, our group observed significant differences in fractional anisotropy in frontal white matter based on the athletes' history of concussion, years of football exposure, and playing position (**Figure 2.2**). These differences were greatest in the forceps minor, though no region of interest analysis was performed on limbic system structures. This study was performed with a relatively young population (52-64yrs) that did not display any clinically-apparent cognitive impairment.

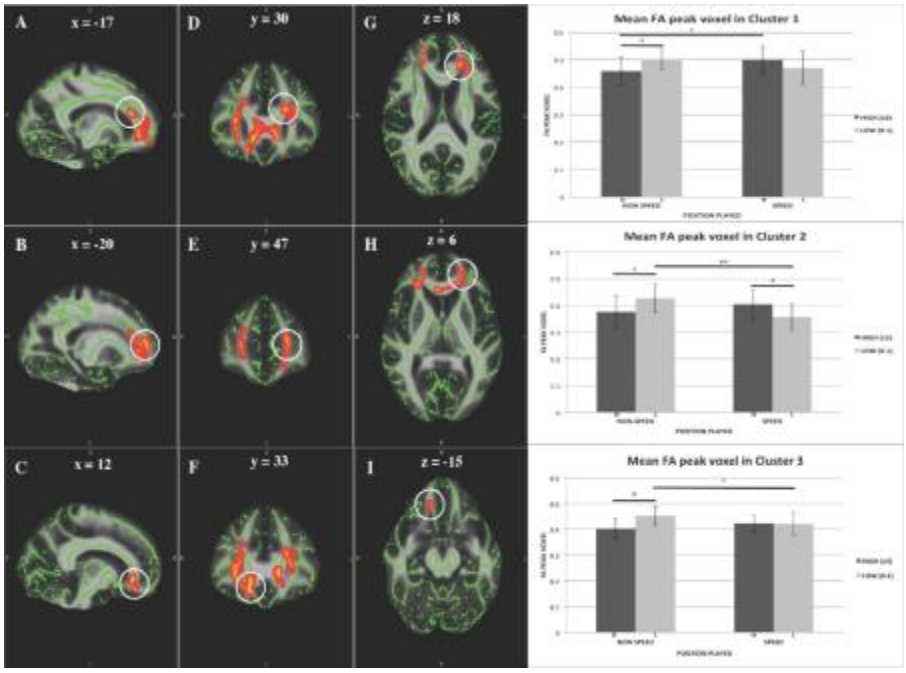


Figure 2.2: Tract-based analysis of the relationship between fractional anisotropy (FA) and concussion history and playing position. The filled FA contrasts are overlaid over the mean FA skeleton (green) and the FMRIB FA template brain. The results are thresholded at p<0.05, and corrected for multiple comparisons using threshold free cluster enhancement. Clusters 1 (A, D, G), 2 (B, E, H), and 3 (C, F, I) are displayed with accompanying graphs showing peak voxel FA values for each of the four groups. *=P<0.05, **=P<0.01.

While DTI can provide valuable information regarding the strength and integrity of white matter connections, it cannot provide information on the function of the gray matter regions that are connected by the white matter tracts. The function of the neurons composing a neural network gives rise to cognitive processes and behavior. In some cases, such cognitive functions and behaviors can be observed outwardly and are what constitute the phenotype of TBI and neurodegenerative disease. This is the grounding motivation for including fMRI in this project; that the neural substrate of the clinical phenotype of CTE may be examined using fMRI. To understand the nature of the fMRI signal and how it can be used in this project, an overview of key concepts in MRI are needed.

Aim 3: Working Memory N-back with Emotional Face Distractors fMRI Paradigm

Functional MRI relies on changes in MR signal produced by the change in the oxygenation of hemoglobin; this is called the blood oxygenation level dependent (BOLD) signal. Because the brain does not have a significant source of stored energy, neurons rely on a constant supply of oxygen and glucose. Without discussing action potentials in depth, the major rectifying ion pump is the Na^+/K^+ pump, which requires adenosine triphosphate (ATP, the major energy currency of cells). When action potentials occur in a brain region, there is a rise in demand for ATP, which is largely produced through glycolysis, citric acid cycle, and electron transport chain. These processes require oxygen and glucose; the former is carried by hemoglobin in red blood cells. Because of the increased demand for ATP, and therefore, oxygen and glucose, there is a need for increased blood flow to the region.

How the brain regulates blood flow in response to neuronal activity is not fully understood. Roles have been implicated for potassium, nitric oxide (NO), and other vasoactive substances released from firing neurons. Additionally, direct neural input into nearby arterioles can cause dilation or constriction through dopamine release. Whatever the molecular

mechanism, there is a well-documented phenomenon of increased blood flow to areas of synaptic activity.

Functional MRI gives information about neuronal activity indirectly through the BOLD response. This change in MR signal is due to the ratio of diamagnetic oxyhemoglobin to paramagnetic deoxyhemoglobin; the former increases signal, while the latter decreases signal. The BOLD signal is affected by changes in cerebral blood flow, cerebral blood volume, and/or the cerebral metabolic rate of oxygen consumption ($CMRO_2$). When neurons fire, there is an initial spike in $CMRO_2$, leading to a rise in deoxyhemoglobin. As this occurs, blood vessels dilate in response to neuronal activity through the release of vasoactive mediators, as discussed in the previous session. The subsequent increase in CBF far exceeds the rise in $CMRO_2$ and the net result is an increase in the ratio of oxyhemoglobin to deoxyhemoglobin. The resultant BOLD signal is the “activation” seen in fMRI.

The advantages of fMRI lie in its spatial resolution (on the order of millimeters), temporal resolution (on the order of seconds; physiological change is rate-limiting), non-invasiveness, and signal-to-noise ratio. These qualities give fMRI excellent functional resolution, or the ability to map physiological variation to mental processes or behavior.

Our group has shown that in an fMRI-based working memory task (without emotionally valent content), former athletes with ≥3 previous concussions had broader functional recruitment of neural circuits than those with 0-1 previous concussions. Despite equivalent performance on the task, the high concussion group (3+) required activation of several additional cognitive resources than the group with 0-1 previous concussions, indicating neural compensation. Furthermore, there were differences in intact white-matter fibers between several regions of interest across both groups, appreciable with DTI, including several within the network activated during the task. These findings indicate that white matter disruption may contribute to the over-activation of frontal and parietal regions during the memory task.

Interestingly, differences in white matter tracts within the limbic system were noted, though this was incidental to the fMRI task as it was not intended to activate limbic regions. These results complement those of another previous study by our group examining players in the same age range who reported subjective recent memory problems, but did not satisfy criteria of mild cognitive impairment. In this study, players with ≥3 previous concussions demonstrated different neural recruitment patterns during relational memory retrieval than those with < 3 concussions (**Figure 2.3**, [101]). These results suggest that multiple concussions may be associated with functional inefficiencies in the working and relational memory networks, possibly as a result of axonal injury from recurrent concussion.

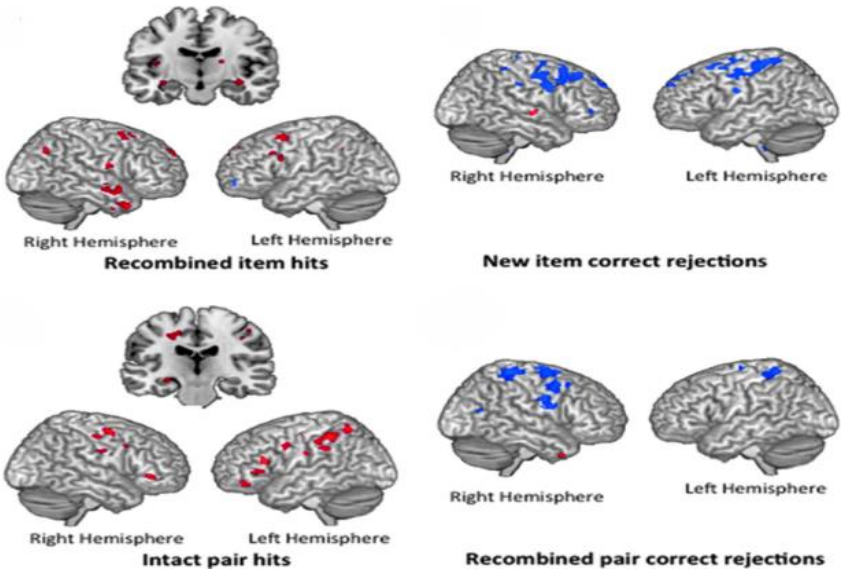


Figure 2.3: Comparison of BOLD response to a relational memory task in former NFL players with variable concussion history. Regions of activation (at $p<0.005$, $k\geq10$) preferentially recruited by persons with increased memory accuracy in the low concussion group relative to the high concussion group (blue) and high concussion group relative to the low concussion group (red).

The paradigm used in the task-based fMRI in this study was an emotional working memory task previously published by Ladouceur et al.[102,103] It is designed to "examine attentional control processes involved in resisting interference from emotionally salient distracters while performing a visual N-back task."[103]. This task has not been used to study former athletes, but it is an appropriate task for this population given the hypothesis of frontolimbic involvement in CTE. Ladouceur et al. observed that young (<18yo) offspring of parents with bipolar disorder had greater right ventrolateral prefrontal cortex activation that healthy controls when facial distractors were present.[108] In another study, they observed that the presentation of fearful faces reduced task performance in children and adolescents with anxiety, suggesting a diminished ability to resist interference from threat-related stimuli as attentional resources are needing to be directed towards task completion (during the 2-back condition).[102] Finally, neutral faces were found to reduce performance in depressed adolescents; interesetingly, this reduction in performance was independent of memory load.[104]

Few studies have examined the frontolimbic network using fMRI in the context of traumatic brain injury. As discussed earlier in this chapter, the frontolimbic network is active during cognitive tasks involving faces expressing emotions and pictures depicting emotionally provocative scenes. Specifically, two major sets of cues have been used in fMRI paradigms previously, the International Affective Picture System (IAPS)[105] and emotional faces.[106] Hariri et al., 2002 demonstrated that the amygdala tends to be more highly activated in response to emotional faces than the IAPS set of cues.[107] Moreover, the amygdala is thought to play a role in providing emotional bias signals in facial processing.[108] This suggests that the amygdala is partly responsible for the interpretation of the emotional valence and content for facial features. In particular, fearful faces have been shown to be more activating than angry faces.[109]

Because facial cues, particularly fearful faces, have been shown to be highly activating with respect to the amygdala, they will be incorporated into the fMRI paradigm in the current

study. In Aim 2 we will use an fMRI task of working memory with emotionally salient content. The design of the paradigm will follow Ladouceur et al, 2009 and 2013[102,103] and is explained in detail in Chapter 3. This paradigm enables examination of the frontoparietal and frontolimbic networks, two distinct neural networks involved in attentional control, working memory, and emotional processing. The task requires subjects to engage prefrontal regions underlying executive control to maintain attention on the task in the presence of emotional distractors. We postulate that recurrent concussions and repetitive head impacts result in frontolimbic neurodegeneration which will impair attentional control. We expect the frontoparietal network, which is activated in working memory tasks,[110-112] to be similar between groups when distractors are not present, but that there will be greater activation of task irrelevant regions will in the impaired group in the presence of the distractors, reflecting a lack of inhibitory control of the limbic system by the prefrontal cortical areas.

Summary

If our hypotheses are correct, frontolimbic dysfunction may be recognized as a unique hallmark of cognitive decline secondary to repetitive head trauma that is detectable prior to diagnosis of dementia. These results would have clinical implications for athletes at risk for recurrent concussion in sports, from youth through professional levels, as well as military personnel suffering from TBI, who are also at greater risk for developing neurodegenerative disease.[113] In the population with this history of head trauma, characterizing neuropsychiatric symptomatology may provide insights for development of more appropriate screening tools and therapeutic interventions. Imaging biomarkers may have use as markers of treatment efficacy in clinical trials.

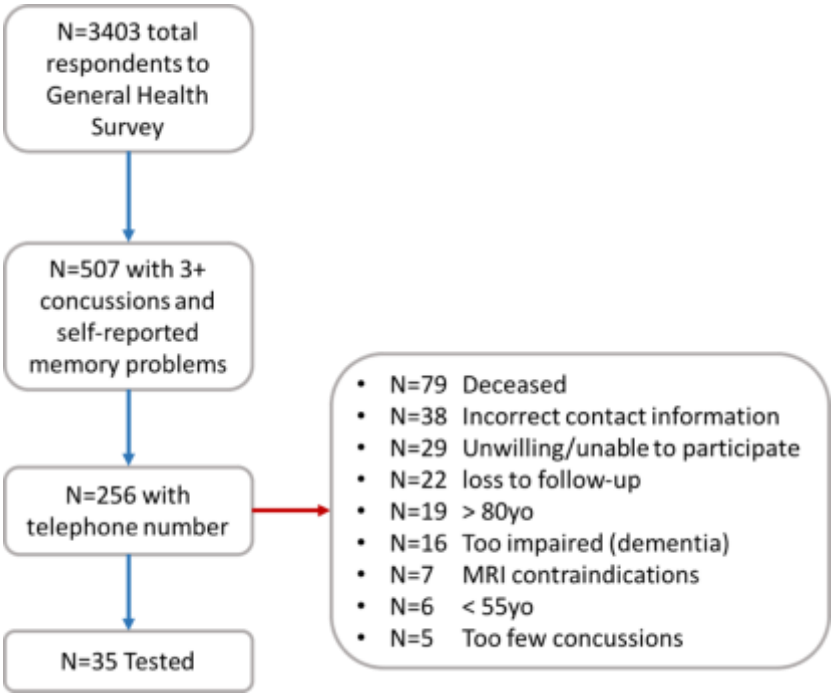
CHAPTER 3: METHODS

Overview

The purpose of this case-control study was to examine early cognitive and behavioral impairments in former professional football players with a history of recurrent concussion. This group has been shown to be at higher risk than the general population for depression, mild cognitive impairment, and CTE. In vivo evidence of the onset and progression of CTE is lacking. Identifying objective measures of impairment in the critical prodromal period of MCI or MBI (or both) will inform diagnostic and prognostic criteria for the clinical assessment of such early impairments.

Recruitment

From January 2016 to June 2017 we recruited a sample of 35 former NFL players from a registry maintained by the Center for the Study of Retired Athletes (CSRA). This registry was initially created for the purposes of the General Health Study, a mailed survey study of former NFL players covering aspects of physical, mental, and psychological health. The total respondents to this survey, which was continued in various modified forms until 2012, includes approximately 3403 subjects. Our center has recruited from this population over a number of years with high response rates of approximately 70%. [2,42,101] Of the ~3,400 former NFL players, 1,282 report more than two prior concussions and 507 self-reported memory/cognitive problems at some point over the period of 2001-2012. Of these 507, a telephone number was available for 257. Of these, 79 were deceased and 38 had incorrect contact information and were unable to be reached. Of the 150 successfully contacted former athletes, we recruited 36 into the study. A diagram of the recruitment is provided in **Figure 3.1.**


N=3403 total respondents to General Health Survey
N=507 with 3+ concussions and self-reported memory problems
N=256 with telephone number
N=35 Tested
N=79 Deceased
N=38 Incorrect contact information
N=29 Unwilling/unable to participate
N=22 loss to follow-up
N=19 > 80yo
N=16 Too impaired (dementia)
N=7 MRI contraindications
N=6 < 55yo
N=5 Too few concussions

Participants

All participants gave both verbal and written informed consent in accordance with the requirements of the Institutional Review Board. The Modified Telephone Interview of Cognitive Status (mTICS) was administered to all subjects. Twelve subjects with a score of <20 were excluded – this likely represents more than mild impairment. Four subjects either self-reported being diagnosed with dementia or a spouse, caregiver, or guardian reported such diagnosis or equivalent advanced impairment preventing travel (e.g. lives in assisted care home).

Subjects must have played a minimum of three seasons at each of the following levels of football: high school, college, and professional (minimum of nine years of football exposure). Furthermore, they must have reported at least three concussions in their lifetime. A standardized definition of concussion[114] was provided to each subject before asking how many concussions they experienced over the course of their lifetimes. The definition provided was:

> A concussion is a blow to the head followed by a variety of symptoms that may include any of the following: headache, dizziness, loss of balance, blurred vision, "seeing stars," feeling in a fog or slowed down, memory problems, poor concentration, nausea, or throwing-up. Getting "knocked out" or being unconscious does *not* always occur with a concussion.

In two cases, subjects reported fewer than three concussions, but reported three or more events in which concussion symptoms were experienced (e.g. headache, "feeling not right," "seeing stars," etc.), stating that they did not consider these events to be "true" concussions. Based on the criteria in the provided definition of concussion, such events were included in their total self-report and they were considered eligible for the study.

For all subjects, inclusion criteria were male sex and age between 55 and 80. For all subjects, exclusion criteria include any diagnosis of dementia including probable Alzheimer's disease, frontotemporal dementia, vascular dementia, dementia with Lewy bodies, or Creutzfeldt-Jakob disease; any contraindications for magnetic resonance imaging including, claustrophobia, pacemaker, surgical clips, pins, plates, screws, metal sutures, wire mesh, or weight exceeding 300 pounds; history of moderate or severe TBI resulting in hospitalization;

severe psychiatric disease such as bipolar or schizophrenia; diagnosis of amyotrophic lateral sclerosis, multiple sclerosis, or history of a major stroke. In this sequence of telephone screeners, if any exclusion criteria were violated, the remaining instruments were not administered. Once inclusion/exclusion criteria were met, enrolled subjects were brought to the University of North Carolina at Chapel Hill for assessment.

Classification of Participants

Classification of impairment was determined during the in-person visit. Subjects were classified as having either mild cognitive impairment (MCI), mild behavioral impairment (MBI), or both, or neither. To assess MCI, a trained research assistant administered the Clinical Dementia Rating (CDR). The CDR is a structured interview with the subject and an informant and covers both subjective and objective assessments of cognition and daily function. The benefit to using the CDR is the qualitative approach to assessing the presence and impact of cognitive impairments on daily function. The CDR is scored from 0 to 3 with a score of 0.5 representing probable mild impairment and scores of 1-3 representing various stages of dementia. Subjects with a CDR score of 0.5 were classified as having MCI. Two subjects scored a 1 on the CDR and were excluded from the analyses as this reflects greater than mild impairment with subsequent loss of independent function A limitation of the CDR is that it is biased towards detecting functional impairments in those with Alzheimer's disease. We classified MBI using the NPIQ. Subjects with two or more symptoms of greater than mild severity and which cause more than mild distress in the informant were classified as having MBI.

This project consisted of a telephone-based recruitment and a single, in-person assessment. Potential subjects were contacted by telephone and verbally consented. The functional activities questionnaire was used to assess independent daily function, an MRI screening form was used to assess any contraindications to MRI scanning, and finally, the mTICS assessed cognitive function. A cut-off score of 30 on the mTICS has been shown to be

at least 78.6% sensitive and 85.5% specific in identifying MCI from those with asymptomatic cognition.[115,116] However, other studies have shown the mTICS to be sensitive to age and educational attainment.[117,118] As such, we decided against using a hard cut-off for defining probable memory impairment given the high educational attainment and variable age of our cohort. Instead, we relied on the in-person visit for classification, as described above.

Aim 1: Neuropsychological and Neuropsychiatric Assessment.

In Aim 1, the National Institutes of Health (NIH) Cognition Toolbox will be used to assess several domains of cognitive function including language, attention, memory (working and episodic), executive functioning (set-shifting and self-monitoring), and processing speed. This battery is augmented with several paper and pencil cognitive tests which assess overlapping domains of cognitive function. **Table 3.1** provides an overview of the assessments and instruments used in Aim 1.

Table 3.1: Overview of instruments and methods. Bolded items are primary outcomes.

Aim	Instruments	
Recruitment	Verbal Consent	
	MCI survey	
	MRI screening form	
	Functional Activities Questionnaire	
	Modified Telephone Interview of Cognitive Status	
1	**NIH Toolbox Cognition Battery**	Interpersonal Reactivity Index
	Picture Vocabulary	Medications and Co-Morbidities Sheet
	Oral Reading Recognition	Head Impact Exposure Estimate
	List Sorting	**Positive and Negative Affect Schedule**
	Picture Sequence Memory	Short Form 36
	Pattern Comparison	Cognitive Symptom Checklist
	Flanker Inhibitory Control	Circadian Sleep Inventory
	Dimensional Change Card Sort	NIH Emotions Battery
	Buss-Perry Aggressiveness Questionnaire	PROMIS Emotional well-being
	Beck Depression Index	NACC Medications and Health history
	Generalized Anxiety Disorder – 7	Shipley Vocabulary
	Mini Mental State Exam	**Trails A and B**
	Symbol Digit Coding	Clinical Dementia Rating
	Patient Health Questionnaire-9	<u>Informant surveys</u>
	CERAD word list learning and **delayed recall**	Neuropsychiatric Inventory Questionnaire
	Headache Impact Test	**Behavioral Inhibition Scale**
	Alcohol Use Disorders ID Test	Functional Activities Questionnaire
	Pittsburgh Sleep Quality Index	Circadian sleep inventory (part 1)
	Controlled Oral Word Association Test	Cognitive Symptom checklist

Aims 2 and 3: Neuroimaging Overview

All imaging was conducted in the Biomedical Research Imaging Center at the University of North Carolina at Chapel Hill on a Siemens Biograph mMR 3T MR-PET scanner. The following sequences were collected for all subjects: high resolution structural (T1 and T2), diffusion-weighted imaging, resting-state functional, and emotional working memory functional. A subset of subjects was injected with a tau PET ligand and steady state uptake was collected for 30 minutes at the start of the scan while the structural and resting state scans were acquired. A sagittal T1-weighted magnetization prepared rapid gradient echo (MPRAGE) anatomical sequence was acquired with a voxel size of $1x1x1mm^3$ over 192 slices with TR/TE = 1900ms/2.26ms. This anatomical scan was used for registration of the diffusion-weighted and functional MR volumes. Additionally, a T2-weighted sequence was acquired with the same voxel size and number of slices with TR/TE=3200ms/402ms. This sequence was used to improve the FreeSurfer parcellation and segmentation described below.

The T1- and T2-weighted anatomical DICOM data were converted to NIfTI file format by the MRIcron tool, *dcm2nii.* These data were processed using the FreeSurfer *recon-all* command, with the T2-weighted image used to improve definition of the pial surface. The FreeSurfer segmentation and parcellation processing stream includes the pre-processing steps of removing non-brain voxels (skull-stripping), registration of the T1- and T2-weighted images, cortical surface reconstruction, cortical and subcortical segmentation, and volume, cortical thickness, and surface area estimation.

Quality control of automated segmentation and parcellation was performed though visual inspection of the estimated pial and grey-white matter interface. Representative images of these surfaces are shown in **Figure 3.2**. Based on previous literature[119,120] showing strong agreement between the estimated volumes of hand-drawn regions of interest (traced by trained radiologists) and those automatically defined by FreeSurfer, we chose not to manually refine the

region-of-interest boundaries that were approximately correct. Instead, images with obviously incorrect boundaries (e.g. a pial surface drawn through a ventricle) were corrected per the FreeSurfer tutorial on quality assurance.

Missing Data

One subject in the MCI group could not complete any imaging sequence due to claustrophobia; his clinical data are included in the Aim 1 analysis. One subject in the MCI+MBI group did not complete the fMRI sequence because of scanner hardware problems. Another subject in the asymptomatic group was unwilling to complete the fMRI sequence because the scanning session ran much longer than usual, again due to scanner hardware issues. Data for one subject in the asymptomatic group were unable to be processed using the FreeSurfer processing stream because of significant global atrophy (**Figure 3.3**). This subject's imaging data were discarded as the analysis of diffusion-weighted and functional images depends on the FreeSurfer surface reconstruction and the mapping of anatomical priors onto the processed T1 image. One subject in the Asymptomatic group had low signal-to-noise ratio for one of the three diffusion-weighted sequences (described below), but his data were still used for the Aim 2 analyses. **Table 3.2** provides an overview of the available subject data by aim.

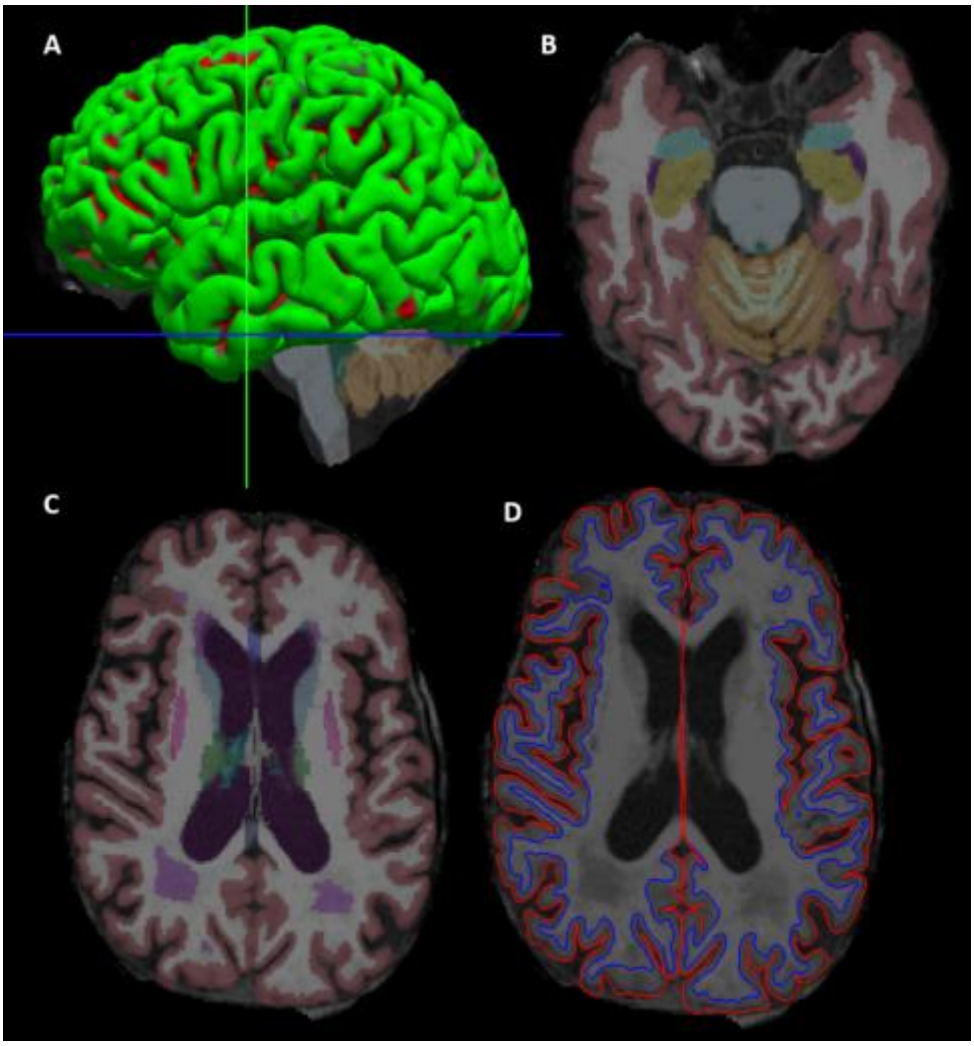


Figure 3.2: Representative example of FreeSurfer surface reconstruction and segmentation. A) 3D view of left hemisphere cortical surface; B) subcortical segmentation showing amygdala (blue) and hippocampus (yellow); C) subcortical segmentation at more superior slice; D) surface reconstruction with grey-white interface (blue) and pial surface (red).

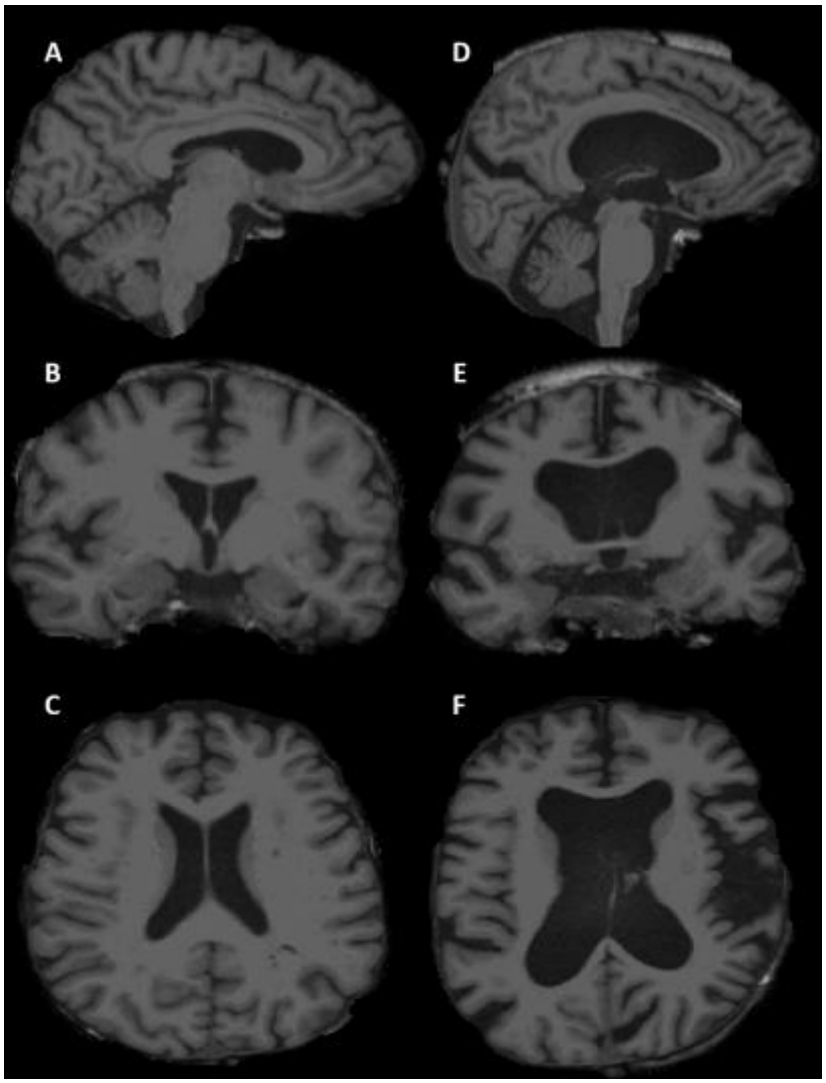


Figure 3.3: Subject with global atrophy whose data was discarded from imaging analyses. A-C) age-matched subject without major atrophy, D-F) subject with signiticant atrophy precluding FreeSurfer analysis.

Table 3.2: Available data by aim.

	Aim1	**Aim 2**	**Aim 3**
Asymptomatic	15	14	13
Impaired	18	17	15
Total	33	31	28

Aim 2: Diffusion-Weighted Imaging Acquisition, Processing, and Analysis

Three sets of diffusion-weighted images (DWIs) were acquired with b values of 300 s/mm^2 (8 directions), 700 s/mm^2 (32 directions), and 2000 s/mm^2 (64 directions). These images were acquired with isotropic 2mm^3 voxels, 74 slices, TR/TE = 5860ms/99.8ms. For every 8 diffusion-weighted images acquired, one unweighted "baseline" (b=0) image was acquired, giving a total of 13 total baseline images and 104 diffusion-weighted vectors.

Quality Control and Preprocessing

For each subject, DICOM data from each of the three DWI sequences were converted to NIfTI format and concatenated into one four-dimensional volume. The b-value and b-vector files corresponding to each of the sequences were combined and the unit-normed b-vectors were scaled according the maximal b-value. The concatenated NIfTI file was converted to Nrrd format using DWIConvert 4.4.0. The Nrrd files were then processed using DTIPrep 1.2.4, a software program that runs an automated quality control algorithm to remove images with motion, vibration, and intensity artifacts. In addition to quality control, DTIPrep also performs the preprocessing steps of eddy current and motion correction and adjustment of gradient vectors following these steps. In brief, the quality control protocol within DTIPrep includes several steps critical for ensuring high-quality DWIs. First, general image information checks are performed on the dimensions, spacing, and orientation of the raw DWIs. Next, the diffusion encoding information is checked, including gradient orientation and b-values. Inter-slice brightness artifacts (changes in intensity between slices of a single DWI volume) are removed, followed by removal of motion and "venetian blind" artifacts (i.e. interlace correlation analysis). Motion-correction is performed on the resultant data using an iterative average of the baseline images as the registration target, rather than registering the images to a single volume. The software also corrects the diffusion gradient directions to account for motion and eddy current correction.

The final step includes reconstruction of diffusion tensor data and computation of fractional anisotropy and mean diffusivity using a single tensor model fitted using weighted least-squares. Following DTIPrep, the cleaned DWIs were visually inspected for any motion artifacts that were not removed by the automated process. 40% of one subject's b=2000 DWIs were discarded due to poor signal-to-noise (**Figure 3.4**), which was caused by improper fit of the head coil during the scan, which came unmoored during the b=2000 sequence. Quality controlled diffusion data for all other subjects were retained with the mean percentage of volumes removed being 19.45%.

The cleaned DWIs were then processed using FreeSurfer's diffusion-imaging analysis pipeline, Tracts Constrained by Underlying Anatomy (*TRACULA*).[121] This tool uses native-space global probabilistic tractography with anatomical priors taken from the segmentation and parcellation of the T1-weighted anatomical scans for each subject. The *TRACULA* processing stream proceeds in three parts, 1 – distortion correction and registration, 2 – tractography using a ball-and-stick model, and 3 – reconstruction of white-matter pathways. First, *TRACULA* computes head motion over the DWI volumes and performs intra-subject registration of the DWI to T1-weighted anatomical scan using FreeSurfer's affine registration tool, *bbregister*, which uses the cortical surface reconstruction to improve registration accuracy. Then, individual T1-weighted images are registered to a common template space, the 1mm-resolution MNI-152 atlas using FreeSurfer's non-linear registration method, *CVS* (Combined Volume and Surface registration). Cortical and white-matter masks are then created from the segmentation and parcellation of the T1-weighted anatomical scans that have already been processed using *recon-all,* as described above. Lastly, anatomical priors for white matter pathways are computed for 18 pathways. Here, we focus on the components of the frontolimbic system, the uncinate fasciculus, cingulum bundle (cingulate gyrus endings and the angular bundle), and the corpus callosum (forceps major and minor).

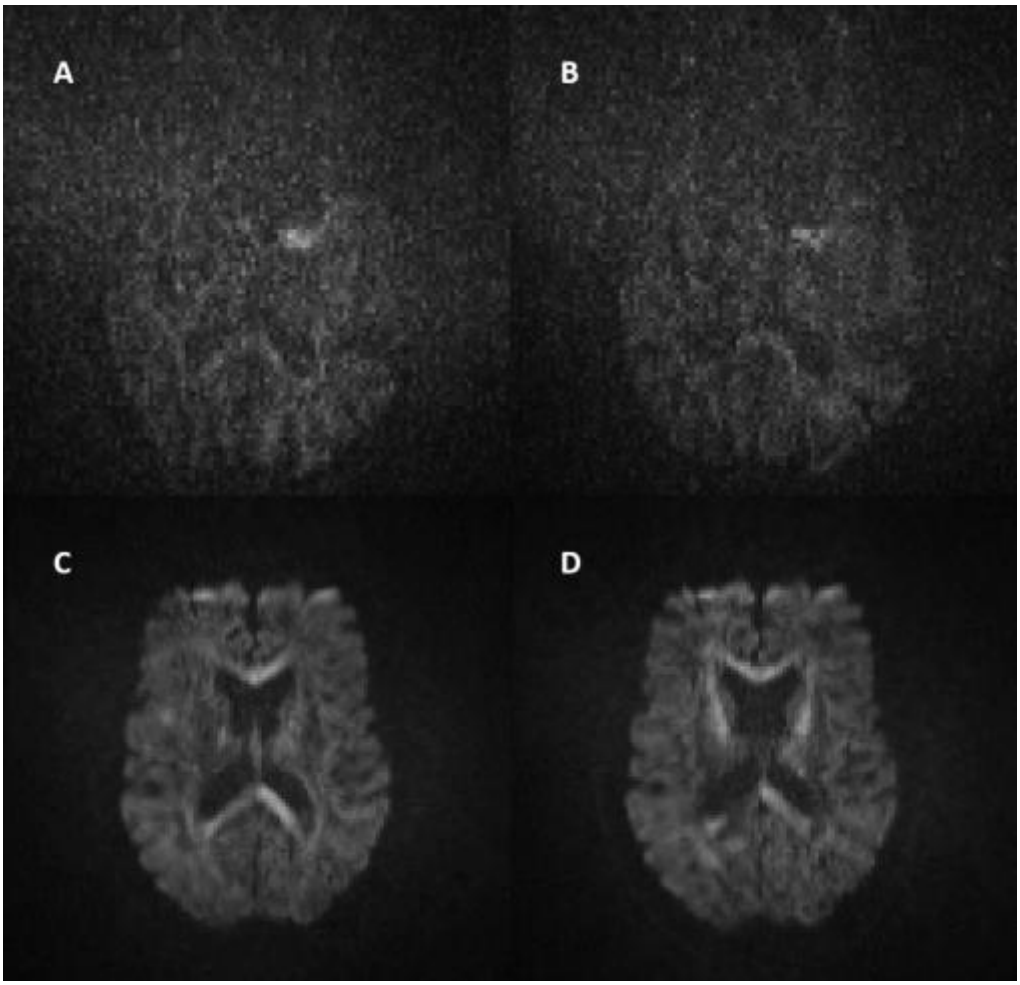


Figure 3.4: Raw diffusion images from a subject with poor signal-to-noise. A-B) discarded b=2000 images from subject #5; C-D) high quality b=2000 images from subject #36.

The second step of *TRACULA* involves probabilistic tractography using FSL's Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques with crossing fibers (BEDPOSTx).[122,123] The BEDPOSTx tool uses Markov Chain Monte Carlo sampling to model a distribution of diffusion parameters at each voxel. A ball-and-stick model is used to model multiple diffusion compartments within each voxel, with the stick modeling anisotropic diffusion and a ball representing the isotropic component. This model is able to incorporate multiple fibers within a voxel, improving the fit of diffusion parameters (**Figure 3.5**). Lastly, pathways are reconstructed from the fitted diffusion parameters using anatomical priors derived from an atlas of manually drawn white matter regions of interest (**Figure 3.6)**. The fitting of these anatomical labels to the subject's data is automated and involves placing control points and drawing streamlines through the control points and applying a probability function to each streamline (**Figure 3.7)**.

Once the pathways are reconstructed, tensor parameters of axial, radial, and mean diffusivity and fractional anisotropy are extracted for each pathway of interest. These parameters can be derived from the center streamline of the pathway, the streamline with the maximum probability of being in the pathway, or a weighted or unweighted average of all streamlines composing the pathway with weights derived from the probability assigned to each streamline from the *TRACULA* reconstruction. For our analyses, we chose to use the weighted average of mean diffusivity (MD), axial diffusivity (AD), radial diffusivity (RD), and fractional anisotropy (FA) for each of our four regions of interest: the forceps minor and major, cingulum (including cingulate gyrus end and the angular bundle), and the uncinate fasciculus. The weighted means for each region were extracted and exported to a spreadsheet and analyzed in R.

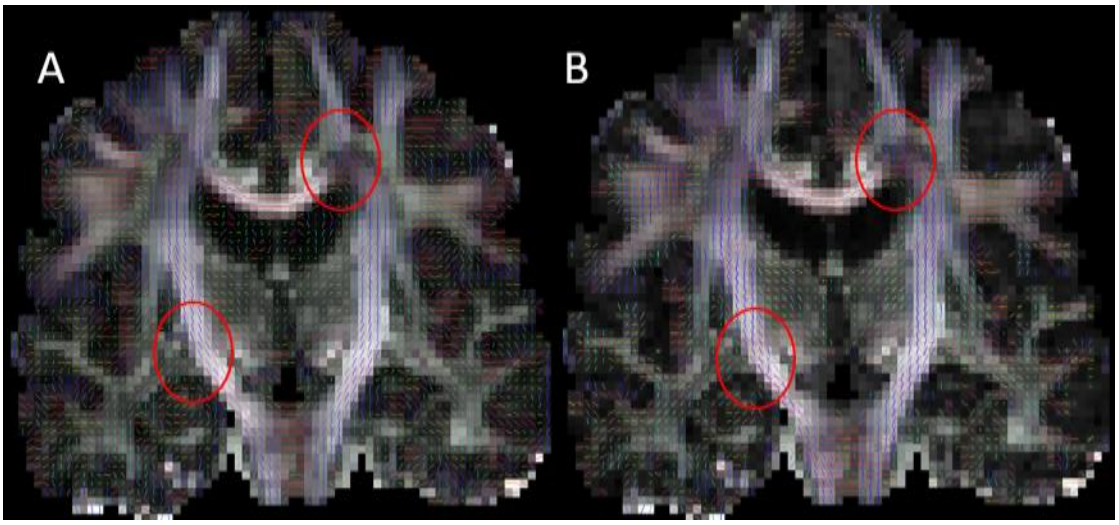


Figure 3.5: Example of crossing fiber modelling using BEDPOSTx. The dominant diffusion direction is displayed in A with red cricles highlighting areas with crossing fibers, as visualized in B.

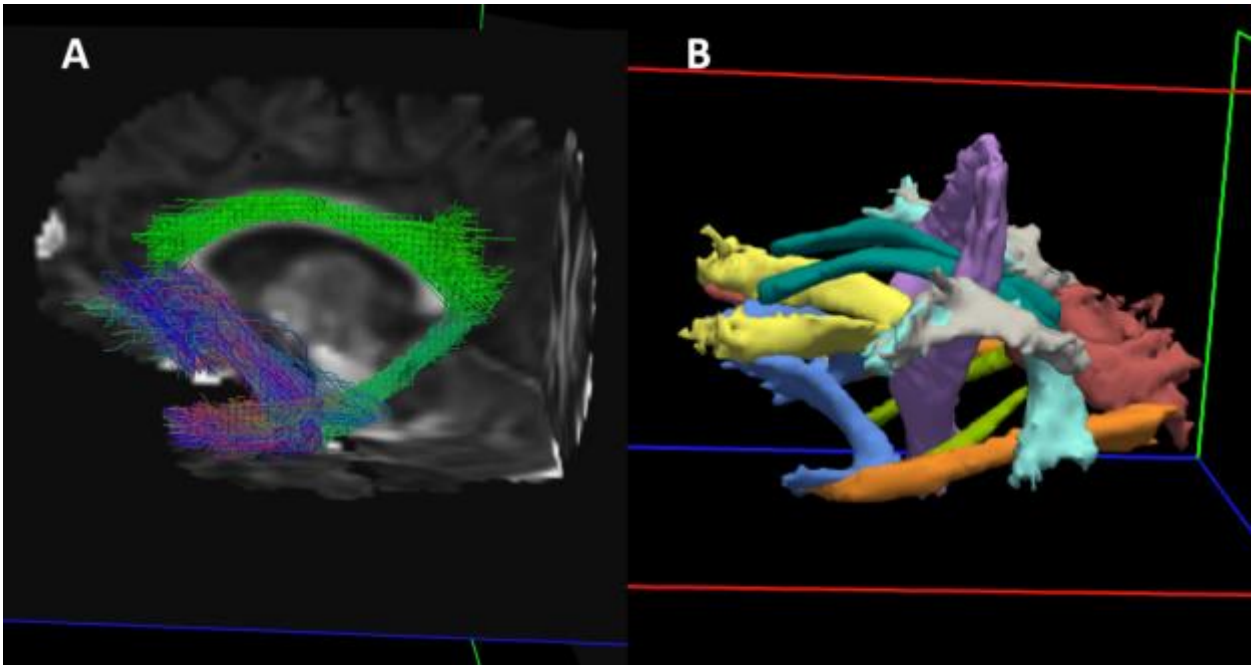


Figure 3.6: Example of reconstructed white matter pathways in *TRACULA*. A) Streamlines for the left uncinate fasciculus (blue), left cingulum – cingulate gyrus ending (green, center), and cingulum – angular bundle (teal, bottom right). B) Volumetric representation of all pathways reconstructed by *TRACULA* with threshold of 60% probability that the voxel lies within the region of interest.

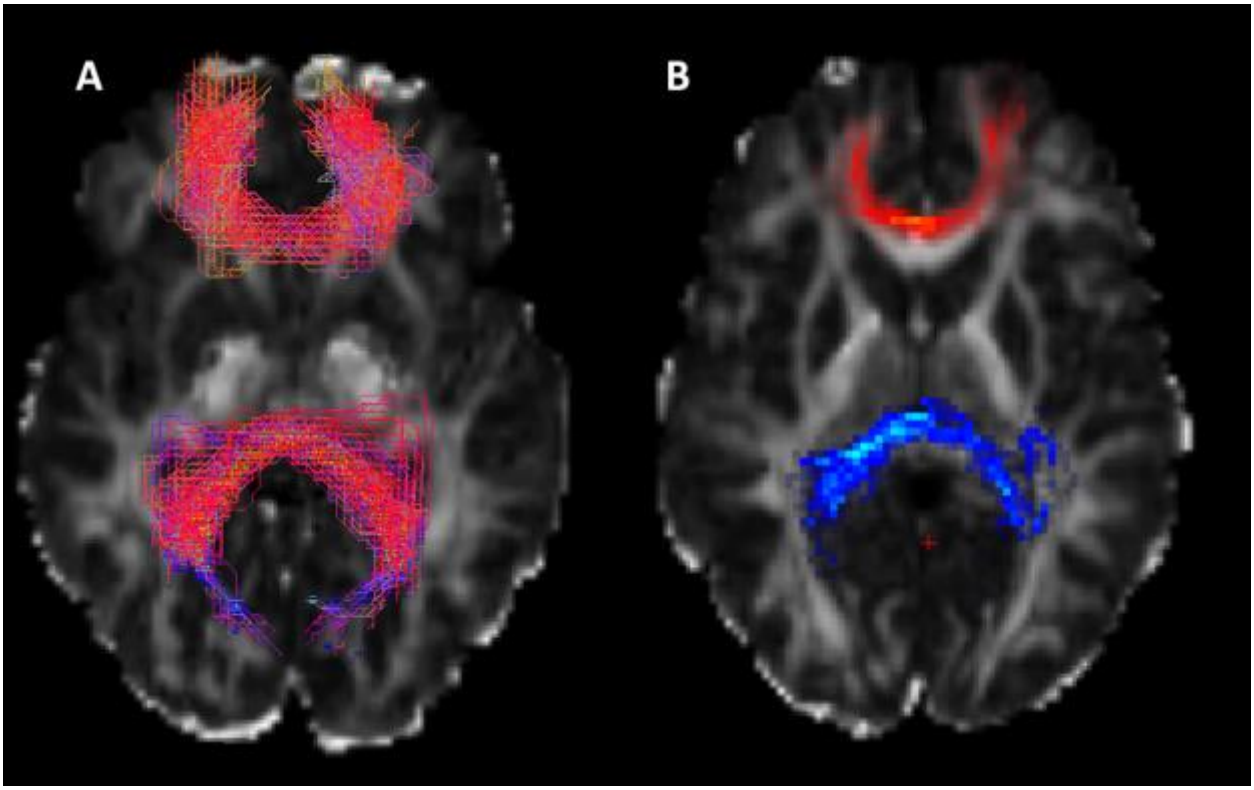


Figure 3.7: Example of streamlines with underlying probability function associated with path reconstruction. A) Streamlines for the forceps minor (top) and forceps major (bottom). B) Probability distribution of voxels within the forceps minor (red) and forceps major (blue).

Neurite Orientation Dispersion and Density Imaging (NODDI)

In addition to tensor-based diffusivity metrics, we estimated the tissue microstructure indices of orientation dispersion (OD), intracellular volume fraction (ICV), and isotropic volume fraction (ISO) using NODDI.[124] In the NODDI model, water diffusivity is partitioned into three compartments: 1 – intracellular water with anisotropic restricted diffusion, 2 – CSF water with isotropic Gaussian diffusion and 3 – extracellular water with anisotropic hindered diffusion. These three compartments are summarized by the indices OD, ICV, and ISO, respectively. These indices range from 0 to 1. For OD, a value of 1 indicates completely parallel diffusion, while 0 indicated completely random diffusion orientation. By contrast, the DTI model accounts for only one diffusion compartment. The more complex NODDI model allows for better characterization of the diffusivity signal at each voxel, and may provide insights into the underlying cellular processes affecting the diffusivity signal. Thus, additional information is gained using a combined approach. A representative subject's DTI and NODDI data are shown together in **Figure 3.8**.

To fit the NODDI indices on the quality-controlled, preprocessed DWIs, we used accelerated microstructure imaging via convex optimization (AMICO).[125] This python-based adaption of the original Matlab NODDI toolbox reduces the fitting time by several fold. One-way analysis of covariance (ANCOVA) was used to compare our study groups with age entered as a covariate. Normality of residuals and homogeneity of variances were assessed using the Wilk-Shapiro test and Levene's test, respectively.

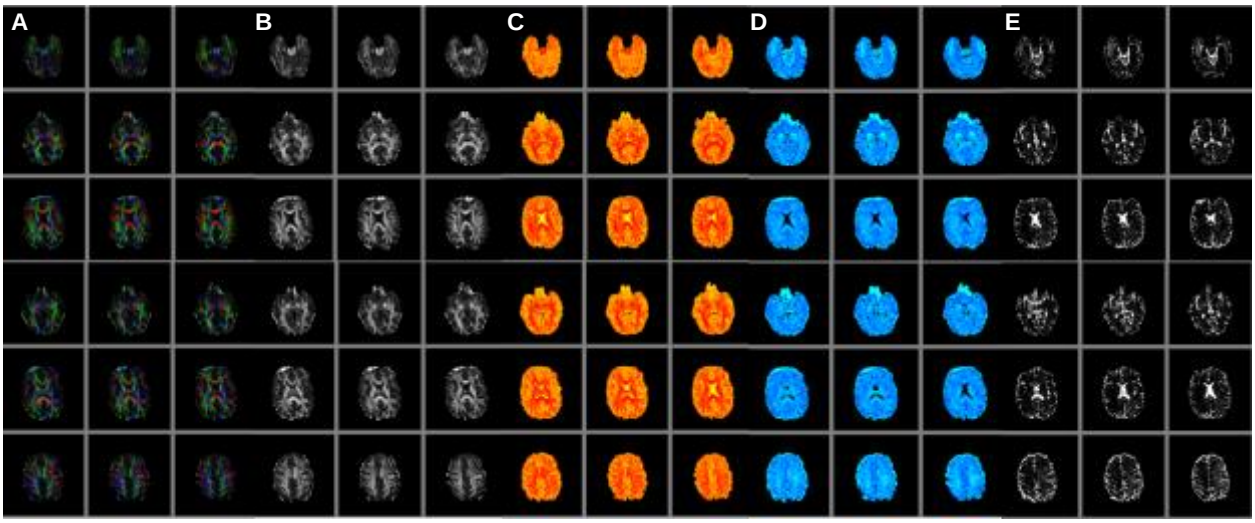


Figure 3.8: DTI and NODDI images from a single subject. A) Color FA map with color-labeled orientation directions, B) Fractional Anisotropy, C) Orientation Dispersion Index, D) Intracellular Volume Fraction, and E) Isotropic volume fraction.

Aim 3: Functional MR Image Acquisition, Processing, and Analysis

Acquisition

Functional MR data were acquired in one run of approximately 15 minutes on a Siemens Biograph mMR with voxel size $3mm^3$ over 47 slices with TR/TE=3000/25ms. Between each slice, a 0.5mm skip was added to ensure full coverage of the cerebrum.

Prior to entering the scanner, subjects were familiarized with the in-scanner working memory task. This familiarization session entailed an overview of the n-back which included practice runs for each level (0-, 1-, and 2-back) without visual distractors. Subjects were given feedback on their performance until they were able to complete each level with 100% accuracy. Following practice on the task, the subjects were then told there would be faces on either side of the letters in the middle of the screen, but that they should not pay attention to the faces. The subjects were shown examples of screens with letter or fixation cross and faces expression either a neutral or fearful expression. Subjects were also asked to identify the emotion being expressed; every subject was able to successfully identify neutral and fearful faces upon first request. This familiarization session, including practice, typically lasted between 5-10 minutes.

For subjects undergoing the additional concurrent PET sequence, intravenous access was obtained and a dose of 185MBeq of THK-5351 was injected 30 minutes prior to the scan start time. This injection occurred after the familiarization session. All subjects were instructed to empty their bladder before the start of the scan. The total scanning time was approximately 60 minutes.

Dr. Ladouceur kindly provided an E-Prime script to run this paradigm, which was modified to include a 1-back level to the n-back task and remove the happy faces distractor condition (for the sake of reducing overall time in the scanner and performing the task). The task followed a blocked design wherein subjects were presented with a pseudorandom sequence of letters flanked by two emotionally expressive faces. In the most basic condition (0-back),

subjects were told to press a button when they saw a specified letter (“M”); in the low cognitive load condition (1-back), subjects were told to press a button when they saw a letter repeated back-to-back (e.g., “A”-“A”); at the higher attentional load condition (2-back), they are asked to press a button when a letter is the same as the one presented two stimuli earlier (e.g. “A”-“B”-“A”). For each attentional load level, there are two distracter conditions, neutral faces and fearful faces, and a condition without facial distractors. Each trial consists of a letter presented in the middle of the screen with either no distracter faces, or two identical faces flanking either side of the letter. With each stimulus presentation, a new face was presented. Subjects completed 1 run of 18 blocks, with 12 trials in each block (total duration of 15 minutes). Stimuli were presented for 500ms with the inter trial interval consisting of a fixation cross. The inter-trial interval was jittered with a mean duration of 3500ms. At the beginning of each run, instructions were presented to remind the subject how to complete the task.

During each block, 17 volumes were collected, with the first volume in each block occurring while the instructions for each block were being displayed. E-Prime was used to present stimuli and collect participant responses during the task. Participant response data were exported and used to create timing files for each subject. These timing files contain information on the type of stimulus presented (n-back level and distractor condition), time of stimulus presentation, and participant response, including both accuracy and reaction time. Only accurate trials will be included in the timing files, and thus, the analysis of task activations is restricted to trials in which the subject successfully responded to the task; this ensures the subject was actively engaging with the task with the presupposition that task relevant networks are being activated.

Preprocessing

Functional MRI data were analyzed using FreeSurfer’s Functional Analysis Stream (FS-FAST) 5.1. The FS-FAST analysis is a surface-based analysis, which improves spatial

localization and allows for better visualization of activations on the cortex. [126,127] First, the middle volume of the run was used as the target for registration of all other run volumes. This middle volume was also used for registration to the anatomical T1 scan using FreeSurfer's *bbregister*. Next, the images were intensity normalized to ensure that the scale of voxel intensities over time is equal. The normalized images are then registered to the T1 anatomical scans using a 6 degree-of-freedom registration to the white-grey matter surface (**Figure 3.9**). Motion correction registers all volumes in the functional time-series to the middle volume and an orthogonal matrix is created with the motion correction parameters. The motion parameters files were used later in the analysis as nuisance regressors to account for inter-subject variability in motion during the task, which may be associated with the cognitive state of the subjects. The motion-corrected volumes were slice-time correct to account for the interleaved acquisition of image slices. The pre-processed data are then resampled to three common spaces: the left and right hemispheres of *fsaverage*, the study-specific common space to which the surface data from the FreeSurfer reconstructed anatomical surfaces are resampled, and the MNI 305 template (used for subcortical analyses). Finally, a 6mm full-width, half-maximum smoothing kernel is applied to the data.

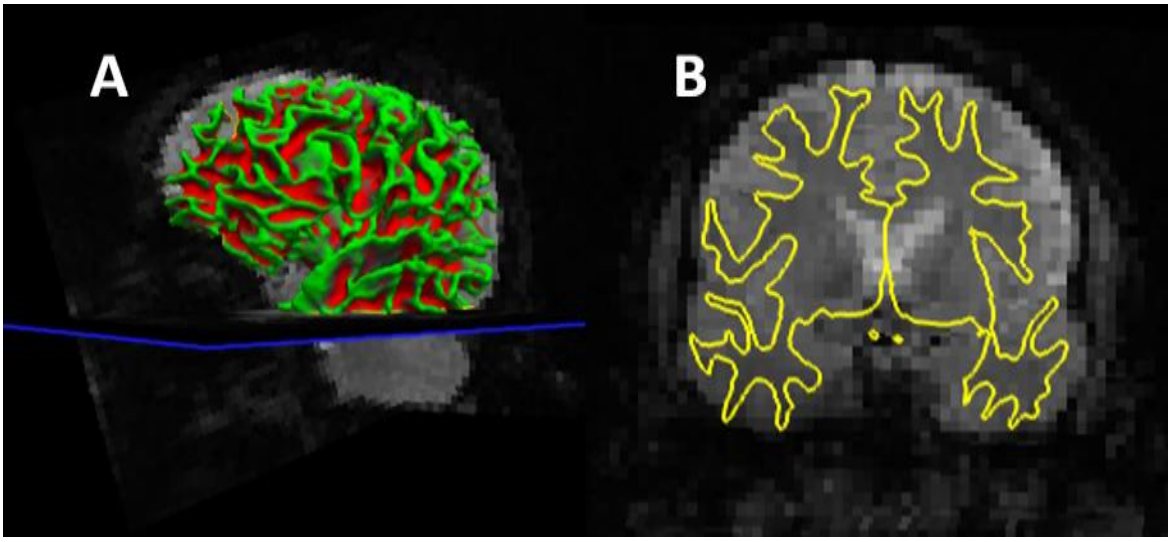


Figure 3.9: Example of functional registration to FreeSurfer reconstructed white matter surface. A) 3D view of the white matter surface overlaid on the middle functional volume of the same subject. B) Coronal slice showing white matter surface in yellow overlaid on middle functional volume.

Analysis

FreeSurfer's general linear model tool, *mri_glmfit,* was used to statistically analyze the fMRI data. A first-level analysis was performed across all subjects (one-sample group mean) with exhaustive contrasts of both the task levels and the distractor conditions. This involved separate contrasts of the n-back levels (e.g. 1-back>0-back, 2-back>1-back, 2>0-back) at each distractor condition and then collapsing across distractor conditions. The same method was employed for the distractor conditions (e.g. neutral faces > no distractor, fearful faces > neutral faces, and fearful faces > no distractor) at each level of the n-back and then collapsing across all n-back levels. In total, 24 first-level contrasts were used. Next, the group-level contrasts compared the asymptomatic group to the impaired group. Finally, a secondary analysis was performed after sub-stratifying the impaired group to those with behavioral impairments and those without and comparing across the three groups (e.g. Asymptomatic > MCI+MBI, Asymptomatic > MCI, MCI > MCI+MBI). In this secondary analysis, an omnibus F-test was used to first determine any significant differences in BOLD signal change. In the case of a significant F-test, the individual contrasts between the groups were compared. Motion parameters and age were entered as nuisance regressors into the group-level analyses.

Comparison for multiple corrections was performed on the statistical maps resulting from the first- and group-level analyses using the FreeSurfer tool, *mri_glmfit-sim*. This correction first sets a voxel-wise alpha of 0.001 for cluster formation (false discovery rate correction) and a cluster-wise correction with an alpha of 0.05. The correction tool is similar to Gaussian random fields (GRF), but uses Monte Carlo simulation with 10,000 iterations rather than analytic equations. This method is based on work described in detail previously.[128]

Data Analysis

All analyses in Aims 1 and 2 were completed in R[20] while the imaging analyses in Aim 3 used the FreeSurfer tool *mri_glmfit*. The outcomes in Aims 1 and 2 will be analyzed using a linear mixed model with group assignment and age as regressors. Heteroscedasticity of the outcomes and normality of residuals will be assessed with Levene's Test and the Wilk-Shapiro test. Where these assumptions are violated, a non-parametric Kruskal-Wallis test will be used in place of the linear model with a regression against age to determine if there is a significant effect of age on the outcome. Where there is a significant effect of age, this will be reported, but for the sake of brevity, non-significant relationships with age will not be exhaustively reported. Task performance data from Aim 3 were also analyzed in R using linear mixed-effect regression with the statistical package *lme4*. Accuracy for each subject was first reduced to a percentage for each n-back level and task condition. The initial analysis of accuracy will assess for main and interactions effects of task level, distractor condition, and impairment status.

The primary analysis for all three aims compares the Asymptomatic and Impaired (MCI or MBI) groups, with a secondary analysis sub-stratifying the MCI into those with MCI+MBI and those with only MCI (comparing Asymptomatic, MCI, and MCI+MBI). One subject was found to have only MBI, this subject was excluded from the secondary analysis as it is unclear to which group he would most appropriately belong. In the secondary analysis, pairwise t-tests will be used if the data are normal or Wilcoxon signed rank if they are not.

CHAPTER 4: RESULTS

Overview

The results of this project are to be disseminated in two manuscripts. Both of the manuscripts are prepared for initial submission to *Neurology.* This journal's audience is primarily clinical neurologists who are knowledgeable of imaging methods, but who are largely non-experts. There is a documented interest in the topic of CTE as many noteworthy studies in the field have been published in *Neurology*. The first manuscript is entitled "White Matter integrity in Former Football Players with Mild Cognitive and Behavioral Impairments." In this manuscript, the diffusion-weighted imaging data of Aim 2 are presented, with the clinical characterization of the sample based on the assessments in Aim 1, including both the paper and pencil cognitive assessments and the NIH Cognition Toolbox. These assessments are easily deployed in a clinical setting and will have relevance to practicing neurologists who see patients with a history of recurrent concussion and concern for underlying CTE.

The second manuscript is entitled "Frontolimbic Neural Recruitment in Former Football Players with Mild Cognitive and Behavioral Impairments." This manuscript centers on the working memory – emotional face distractor functional MRI task. In this paper, we cover the performance on the task and the neural recruitment patterns observed on imaging. While less clinically translatable than diffusion-weighted imaging, this study will provide insight into the functional consequences of mild impairments in highly exposed individuals who show signs of early cognitive or behavioral decline.

White Matter Integrity in Former Football Players with Mild Cognitive and Behavioral Impairments

Introduction

Converging evidence points to an association between recurrent concussive and subconcussive impact exposure and clinical impairments in cognition[2,42] and behavior[1,43] later in life. In some cases, these symptoms may be related to underlying neurodegenerative disease, such as Alzheimer's disease, frontotemporal lobar degeneration, or chronic traumatic encephalopathy (CTE).[6] CTE is a trauma-related neuropathology characterized by perivascular hyper-phosphorylated tau deposition in a patchy distribution that often involves regions in the frontolimbic network, particularly in more advanced stages.[53] A recent study of autopsy-confirmed CTE found 110 out of 111 NFL players had CTE of varying degree.[129] Subsequent commentary has pointed out that 1,300 former NFL players died over the collection period of the study, meaning the minimum prevalence of CTE is 9%, though may be higher.

While the clinical presentation of cognitive decline in those with a known history of head trauma has not been well characterized, the results of observational studies in former NFL players suggests both cognitive and behavioral impairments develop over the course of the disease.[8,45] However, the timing, extent, and progression of these symptoms remain unclear and more studies are needed to better understand the natural history of such decline. This is especially true of the earlier stages of the disease, before subjects are demented. Early recognition of an underlying neurodegenerative process can improve prognosis and identify those who would most benefit from therapies intended to arrest the disease process and restore or maintain functional independence.

Advanced magnetic resonance imaging is sensitive to early changes in neuroanatomy following recurrent concussion, particularly diffusion-weighted imaging (DWI) – a technique that enables quantitative analysis of diffusivity in white matter, a proxy measure of white matter tract integrity. DWI is sensitive to a number of underlying processes in white matter microstructure including trauma, inflammation, myelin-loss, and axonal degeneration. Fractional anisotropy (FA) and mean diffusivity (MD) are diffusion tensor-based metrics sensitive to a loss of integrity in white matter, although lacking in specificity with respect to the underlying cellular process effecting a change in diffusivity. By contrast, neurite orientation dispersion and density imaging (NODDI) allows for the calculation of additional metrics of diffusivity including orientation dispersion (OD), intracellular volume fraction (ICV), and isotropic volume fraction (ISO).[124] OD provides information about the extent of dispersion (e.g. fanning) of white matter tracts while ICV and ISO are related to the density of axons. NODDI has recently been used to study white matter in those with a history of recurrent concussion, finding that in addition to an increase in FA and decrease in MD, there is a corresponding increase in ICV and decrease in OD.[130] Taken together, these results suggest that following concussion, there is a remodeling of white matter that results in greater axonal density and coherence. However, in studies of subjects with more extensive history of concussive impact exposure, the pattern of DTI findings is more similar to those with moderate or severe TBI. In these studies, subjects tended to have lower FA and greater MD,[100,131] suggestive of axonal degeneration and demyelination.[132] NODDI has yet to be used to study subjects with extensive history of recurrent trauma.

This study focuses on former professional football players with and without mild cognitive and behavioral impairments. Using cognitive and psychiatric instruments easily deployed in a clinical setting, we compared cognitive and behavioral functioning between asymptomatic and impaired former players with similar concussive and subconcussive exposure. Using NODDI, we compared white matter integrity within frontolimbic white matter

tracts of interest between the groups. Finally, we examined correlations between tensor and NODDI metrics and our clinical instruments to determine the neural underpinnings of cognitive or behavioral functioning within these samples. We hypothesized that the impaired group would have worse scores on measures of memory, processing speed, and executive function and greater symptomatology related to impulsivity, depression, anxiety, and aggression. We expected these differences in clinical assessments to be positively correlated to white matter integrity within the frontolimbic white matter tracts, namely the cingulate bundle and uncinate fasciculi (UF).

Methods

Participants

From January 2016 to June 2017 we recruited a sample of 36 former National Football League (NFL) players from a registry maintained by the Center for the Study of Retired Athletes (CSRA) at the University of North Carolina at Chapel Hill. Of the approximately 3400 former players in the registry, 1282 report more than two prior concussions and 507 self-reported memory/cognitive problems at some point over the period of 2001-2012. Of these 507, a telephone number was available for 257. Of these, 79 were deceased and 38 had incorrect contact information and were unable to be reached. Of the 150 successfully contacted former athletes, we recruited 36 into the study. All participants gave both verbal and written informed consent in accordance with the requirements of the Institutional Review Board.

Subjects were males between 55 and 80 who played a minimum of three seasons at each of the following levels of football: high school, college, and professional (minimum of nine years of football exposure). Furthermore, they must have reported at least three concussions in their lifetime using a standardized definition of concussion.[114] Exclusion criteria included any diagnosis of dementia including probable Alzheimer's disease, frontotemporal dementia,

vascular dementia, or dementia with Lewy bodies; any contraindications for magnetic resonance imaging, including claustrophobia, pacemaker, unsafe metal implants, or weight exceeding 300 pounds; history of moderate or severe traumatic brain injury resulting in hospitalization; severe psychiatric disease such as bipolar or schizophrenia; diagnosis of amyotrophic lateral sclerosis, multiple sclerosis, or history of a major stroke. In this sequence of telephone screeners, if any exclusion criteria were violated, the remaining instruments were not administered. Once inclusion/exclusion criteria were met, enrolled subjects were brought to the University of North Carolina at Chapel Hill for assessment.

Classification of Mild Cognitive and Behavioral Impairment

Impairment status was determined during the in-person visit. Subjects were classified as having “mild cognitive impairment” (MCI), “mild behavioral impairment” (MBI), or both. All subjects were highly exposed to head impacts throughout their playing careers based on self-reported concussions and the head impact exposure estimate, which accounts for position-weighted exposure hours. To assess MCI, a trained research assistant administered the Clinical Dementia Rating (CDR). Subjects with a CDR score of 0.5 were classified as MCI. Two subjects scored a 1 on the CDR and were excluded from the analyses as this reflects greater than mild impairment. We classified MBI using the neuropsychiatric inventory questionnaire (NPIQ); subjects with two or more symptoms of greater than mild severity and which cause more than mild distress in the informant were classified as having MBI. Those in the asymptomatic group did not meet criteria for either MCI or MBI. It does not, however, preclude subjects who self-report symptoms that are outside of the measurements used to define the groups, namely the CDR and NPQI, which have limits in their applications to the study population that are discussed elsewhere.

Cognitive and neurobehavioral assessment

All subjects completed the National Institutes of Health (NIH) Cognition Toolbox [133] and a focused battery of paper and pencil cognitive assessments in one 2-3 hour session. In addition to the Toolbox, the cognitive battery consisted of the mini mental state exam (MMSE), 10-item delayed word recall, symbol digit coding (SDC), trail making test parts A and B, controlled oral word association test (COWAT), and the Shipley vocabulary test. The psychiatric surveys used were the patient health questionnaire 9-item scale (PHQ-9), Beck depression inventory 2nd Edition (BDI-II), generalized anxiety disorder 7-item scale (GAD-7), Buss-Perry aggression questionnaire (BPAQ), positive and negative affect schedule (PANAS), and the behavioral inhibition scale (BIS), which is completed by an informant.

Imaging Acquisition and Processing

Imaging was conducted in the Biomedical Research Imaging Center at the University of North Carolina at Chapel Hill on a Siemens Biograph mMR 3T MR-PET scanner. A sagittal T1-weighted magnetization prepared rapid gradient echo (MPRAGE) anatomical sequence was acquired with a voxel size of 1x1x1mm^3 over 192 slices with TR/TE = 1900ms/2.26ms. This anatomical scan was processed through FreeSurfer's *recon-all*, a pipeline for parcellation, segmentation, and cortical surface reconstruction and used for registration of the diffusion-weighted volumes. A T2-weighted sequence with same voxel size and slice number with TR/TE=3200ms/402ms was acquired and used in FreeSurfer to improve pial surface reconstruction. Three sets of DWIs were acquired with b values of 300 s/mm^2 (8 directions), 700 s/mm^2 (32 directions), and 2000 s/mm^2 (64 directions). These images were acquired with isotropic 2mm^3 voxels, 74 slices, TR/TE = 5860ms/99.8ms. For every 8 diffusion-weighted images acquired, one unweighted "baseline" (b=0) image was acquired, giving a total of 13 total baseline and 104 diffusion-weighted volumes.

Quality control of diffusion-weighted images was performed using DTIPrep 1.2.4, a program that runs an automated quality control algorithm to remove images with motion, vibration, and intensity artifacts.[134] DTIPrep was also used for the preprocessing steps of eddy current and motion correction and adjustment of gradient vectors following these steps. Following DTIPrep, the cleaned DWIs were visually inspected for any motion artifacts that were not removed by the automated process. 40% of one subject's b=2000 DWIs were discarded due to poor signal-to-noise. Quality-controlled diffusion data for all other subjects were retained with the mean percentage of volumes removed being 19.45%.

The cleaned DWIs were then processed using FreeSurfer's diffusion-imaging analysis pipeline, Tracts Constrained by Underlying Anatomy (*TRACULA*).[121] This tool uses native-space global probabilistic tractography with anatomical priors for 18 white matter pathways; we chose to focus on the components of the frontolimbic system, including the bilateral uncinate fasciculi and cingulum bundles (cingulate gyrus endings and the angular bundle), and the corpus callosum (forceps major and minor). For each pathway, we analyzed the tensor-based metrics of FA and MD and used accelerated microstructure imaging via convex optimization (AMICO)[125] to estimate the NODDI indices of OD, ICV, and ISO.

Data from two subjects (one normal and one with MCI) were excluded from imaging analysis. One subject's data had poor signal-to-noise and another had significant global atrophy, preventing accurate registration and correct anatomical comparison to other subjects.

Statistical Analyses

Statistical analyses were conducted in R. Clinical and imaging outcomes were compared using analysis of covariance (ANCOVA) with impairment status and age as predictors. In the primary analysis, we compare subjects with MCI or MBI to normal subjects. In the secondary

analysis, we compare MCI only, MCI and MBI, and normal with pairwise comparisons after significant omnibus tests. For the secondary analysis, one subject with only MBI, but not MCI was excluded. Where data were heteroscedastic or residuals non-normal, we used non-parametric tests (i.e. Kruskal-Wallis and Wilcoxon rank sum). For the NIH Toolbox, uncorrected standard scores were analyzed.

For DWI metrics, we analyzed the mean of all tractography streamlines in each region of interest weighted by the probability of the streamline being contained in the region; this weighting is intended to reduce partial volume effects. For DTI metrics, we first compared FA and MD between the groups. Where significant differences in tensor metrics were observed between the groups, we compared the OD, ICV, and ISO to further characterize differences in diffusivity.

Results

The normal group was significantly younger than the impaired group ($F_{1,31} = 6.026$, $P = 0.020$) and had significantly more experience at the professional level ($\chi_2^2 = 7.739$, $P=0.006$). Demographic characteristics of the group are given in **Table 4.1** with secondary analysis in **Table 4.2**. The specifics CDR domain scores and NPIQ symptoms reporting for the study cohort are presented in Table 4.3. Because these instruments were used for classification as described in the methods, no statistical comparisons between the groups were performed.

Table 4.1: Demographics of sample comparing Asymptomatic to Impaired (MCI or MBI). Mean (SD) [min, max]

	Asymptomatic (n=15)	**Impaired (n=18)**	***Statistic***	**p-value**
Age (y)	62.5 (4.4) [56, 70]	67.0 (5.8) [56, 77]	6.026 [a]	**0.020**
Education (y)	17.5 (1.9)	16.6 (0.9)	1.582 [b]	0.216
Body Mass Index (kg/m^2)	30.8 (2.5)	30.0 (4.0)	0.381 [a]	0.542
Number of Concussions	8.6 (9.8) [3, 40]	7.9 (4.5) [3, 20]	0.566 [b]	0.452
Lifetime Years of Football	22.3 (6.3) [12, 40]	18.9 (4.0) [12, 27]	3.428 [b]	0.064
Years at Professional Level	12.1 (5.2) [5, 27]	8.1 (2.6) [4,13]	7.739 [b]	**0.006**
Head Impact Exposure Estimate	3379 (1802)	3441 (2840)	0.632 [b]	0.426
Speed Position Players (n)	8	14		
Non-Speed Position Players (n)	7	4		

[a] $F_{1,31}$

[b] Kruskal-Wallis χ^2 statistic

Table 4.2: Demographics of sample comparing Asymptomatic, MCI, and MCI+MBI. Mean (SD) [min, max]

	Asymptomatic (n=15)	**MCI (n=9)**	**MCI+MBI (n=8)**	***Statistic***	**p-value**
Age (y)	63.5 (4.4) [56, 70]	66.6 (6.5) [56, 74]	66.5 (4.9) [61, 77]	2.390 [a]	0.109
Education (y)	17.5 (1.9) [16, 20]	16.9 (1.1)	16.3 (0.7)	2.627 [b]	0.269
Body Mass Index (kg/m^2)	30.8 (2.5) [25.7, 35.6]	29.4 (4.3)	29.7 (3.1)	0.554 [a]	0.581
Number of Concussions	8.6 (9.8) [3, 40]	8.6 (5.6) [3, 20]	7.8 (3.3) [3, 12]	0.761[b]	0.684
Lifetime Football Experience (y)	22.3 (6.3) [12, 40]	18.4 (3.4) [13, 22]	18.4 (3.8) [12, 24]	4.676 [b]	0.096
Professional Level (y)	12.1 (5.2) [5, 27]	8.4 (2.8) [5, 13]	7.1 (2.0) [4, 10]	9.680 [b]	**0.008**
Head Impact Exposure Estimate	3379 (1802)	3292 (2396)	3812 (3529)	0.450 [b]	0.799
Speed Position Players (n)	8	7	6		
Non-Speed Position Players (n)	7	2	2		

[a] $F_{1,31}$

[b] Kruskal-Wallis χ^2 statistic.

Table 4.3: Summary of CDR domains and NPIQ symptoms across study cohort.

	Normal(Asymptomatic) (n=15)			MCI (n=9)			MCI+MBI (n=8)		
Clinical Dementia Rating [a]	*0*	*0.5*	*1.0*	*0*	*0.5*	*1.0*	*0*	*0.5*	*1.0*
Memory	15	0	0	0	8	1	0	6	2
Judgement and Problem solving	12	3	0	3	6	0	2	6	0
Orientation	15	0	0	5	1	3	4	4	0
Community Affairs	15	0	0	5	3	1	5	3	0
Home and Hobbies	15	0	0	8	1	0	4	2	2
Personal Care	15	0	0	9	0	0	0	0	0
Neuropsychiatric Inventory – Questionnaire	*N*	*Mean severity*	*Mean distress*	*N*	*Mean severity*	*Mean distress*	*N*	*Mean severity*	*Mean distress*
Irritability/Lability	3	1.67	2.5	6	1.0	1.0	7	2.0	3.0
Aggression/Agitation	2	1.5	4.0	2	1.0	1.5	5	2.3	3.2
Apathy/Indifference	1	1.0	1.0	2	1.0	1.0	5	2.3	2.0
Disinhibition	2	2.5	3.0	0	N/A	N/A	4	2.0	3.0
Anxiety	1	1.0	2.0	0	N/A	N/A	4	2.0	3.3
Depression/Dysphoria	1	1.0	1.0	1	1.0	0.0	3	2.0	4.7
Motor Disturbance	0	N/A	N/A	0	N/A	N/A	4	1.8	2.5
Elation/Euphoria	2	1.0	0.0	0	N/A	N/A	1	2.0	2.0
Nighttime Behaviors	1	1.0	1.0	1	1.0	1.0	3	2.0	2.3
Appetite/Eating	0	N/A	N/A	1	1.0	1.0	1	3.0	4.0
Hallucinations	0	N/A	N/A	0	N/A	N/A	0	N/A	N/A
Delusions	1	1.0	2.0	0	N/A	N/A	1	2.0	2.0

CDR = Clinical Dementia Rating; NPIQ: Neuropsychiatric Inventory questionnaire; Mean severity ranges from 1 (mild) to 3 (severe); Mean caregiver distress ranges from 0 (not distressing) to 5 (severely distressing).

[a] Presented as number of subjects receiving score of 0, 0.5, or 1.

Cognitive Clinical Assessment

The impaired group had lower mini mental state exam (MMSE) ($\chi_2^2 = 10.358$, P = 0.01) and symbol digit coding (SDC) score ($F_{1,31} = 4.882$, P = 0.027) compared to the normal group (**Table 4.4)**. In the secondary analysis (summarized in **Table 4.5**), the same measures were found to be significantly affected by impairment status. Pair-wise Wilcoxon rank sum tests showed a significant difference between the normal and MCI+MBI groups for both MMSE (P < 0.001) and SDC (P= 0.043). No significant differences were observed between MCI and MCI+MBI for either measure (P = 0.086 and P = 0.085, respectively). No differences were observed between the groups for

With respect to psychiatric symptomatology, we observed a significant difference between the normal and impaired groups on the BDI II ($\chi_2^2 = 6.110$, P = 0.013) and BIS ($\chi_2^2 = 6.593$, P = 0.015). For both of these measures, the normal group had a lower mean score than the impaired group (**Table 4.6**). In the secondary analysis, we observed a significant effect of impairment status on the PHQ-9 ($\chi_2^2 = 7.372$, P = 0.025), BDI II ($\chi_2^2 = 13.704$, P = 0.001), GAD-7 ($\chi_2^2 = 9.818$, P = 0.007), and BIS ($F_{2,29} = 7.891$, P = 0.002). Pair-wise Wilcoxon rank sum tests showed significant differences in each of these measures between the MCI and MCI+MBI groups and the normal and MCI+MBI group (all P < 0.05). No significant differences were observed between the normal and MCI groups for any measure (all P > 0.05) (**Table 4.7**).

Table 4.4: Paper and pencil cognitive assessments comparing Asymptomatic to Impaired (MCI or MBI). Mean (SD).

	Asymptomatic (n=15)	Impaired (n=18)	*F-Statistic*	p-value
MMSE	28.9 (1.2)	27.3 (1.4)	10.358[a]	**0.001**
CERAD delayed recall	5.0 (2.6)	3.7 (1.7)	2.884	0.099
SDC	44.3 (9.0)	37.9 (6.7)	4.882	**0.027**
Trails A (s)	36.4 (10.7)	41.8 (12.0)	1.832[a]	0.186
Trails B (s)	71.1 (22.3)	96.6 (42.1)	3.672[a]	0.057
COWAT	42.0 (13.5)	40.4 (7.7)	0.001[a]	0.971
Shipley Vocabulary	33.9 (2.4)	32.7 (3.8)	1.034	0.317

[a] Kruskal-Wallis χ^2 statistic.
MMSE: Mini Mental State Exam; CERAD: Consortium to Establish a Registry for Alzheimer's Disease; SDC: Symbol Digit Coding; COWAT: Controlled Oral Word Association Test.

Table 4.5: Paper and pencil cognitive assessments comparing Asymptomatic, MCI, and MCI+MBI. Mean (SD).

	Asymptomatic (n=15)	MCI (n=9)	MCI+MBI (n=8)	*F-Statistic*	p-value
MMSE	28.9 (1.2)	27.7 (1.5)	26.6 (1.1)	13.269[a]	**0.001**
CERAD delayed recall	5.0 (2.6)	4.1 (1.8)	3.1 (1.5)	3.641[a]	0.162
SDC	44.3 (9.0)	40.9 (5.2)	34.3 (7.1)	6.912[a]	**0.031**
Trails A (s)	36.4 (10.7)	39.0 (10.7)	45.0 (14.1)	1.450	0.251
Trails B (s)	71.1 (22.3)	89.9 (28.6)	107 (55.5)	4.038[a]	0.132
COWAT	42.0 (13.5)	39.8 (8.2)	41.3 (8.1)	0.114	0.893
Shipley Vocabulary	33.9 (2.4)	33.3 (3.0)	33.1 (3.5)	0.203	0.818

[a] Kruskal-Wallis χ^2 statistic.
MMSE: Mini Mental State Exam; CERAD: Consortium to Establish a Registry for Alzheimer's Disease; SDC: Symbol Digit Coding; COWAT: Controlled Oral Word Association Test.

Table 4.6: Psychiatric symptom surveys comparing Asymptomatic to Impaired (MCI or MBI). Mean (SD).

	Asymptomatic (n=15)	**Impaired (n=18)**	***F-Statistic***	**p-value**
PHQ-9	1.7 (1.6)	3.4 (4.2)	0.215	0.643
BDI-II	2.9 (2.4)	8.4 (7.8)	6.110[a]	**0.013**
GAD-7	0.9 (1.4)	3.8 (4.9)	2.552[a]	0.110
BPAQ total	51.5 (12.3)	51.7 (12.1)	0.001	0.975
PANAS positive symptoms	37.0 (6.5)	34.2 (7.5)	1.273[a]	0.268
PANAS negative symptoms	12.0 (2.2)	15.7 (7.0)	1.910[a]	0.167
BIS (Informant)	15.6 (1.8)	18.0 (3.2)	6.593	**0.015**

[a] Kruskal-Wallis χ^2 statistic.

PHQ-9: patient health questionnaire 9-item scale; BDI-II: Beck Depression Inventory 2nd Edition; GAD-7: Generalized Anxiety Disorder 7-item scale; BPAQ: Buss-Perry Aggression Questionnaire (BPAQ); PANAS: Positive and Negative Affect Schedule; BIS: Behavioral Inhibition Scale (completed by informant).

Table 4.7: Psychiatric symptom surveys comparing Asymptomatic, MCI, and MCI+MBI. Mean (SD) Mean (SD).

	Asymptomatic (n=15)	**MCI (n=9)**	**MCI+MBI (n=8)**	***F-Statistic***	**p-value**
PHQ-9	1.7 (1.6)	1.4 (2.6)	6.0 (4.8)	7.372[a]	**0.025**
BDI-II	2.9 (2.4)	4.4 (5.1)	13.0 (8.5)	13.704[a]	**0.001**
GAD-7	0.9 (1.4)	1.0 (1.7)	7.4 (5.3)	9.818[a]	**0.007**
BPAQ total	51.5 (12.3)	48.1 (9.7)	57.7 (12.5)	1.429	0.256
PANAS Positive	37.0 (6.5)	36.7 (7.3)	30.9 (7.1)	2.291	0.119
PANAS Negative	12.0 (2.2)	13.4 (3.2)	18.9 (9.3)	3.613[a]	0.164
BIS (Informant)	15.6 (1.8)	16.3 (3.0)	19.8 (2.7)	7.891	**0.002**

[a] Kruskal-Wallis χ^2 statistic.
PHQ-9: patient health questionnaire 9-item scale; BDI-II: Beck Depression Inventory 2nd Edition; GAD-7: Generalized Anxiety Disorder 7-item scale; BPAQ: Buss-Perry Aggression Questionnaire (BPAQ); PANAS: Positive and Negative Affect Schedule; BIS: Behavioral Inhibition Scale (completed by informant).

NIH Cognition Toolbox

The normal group had significantly greater uncorrected standard scores compared to the Impaired group for the total composite score ($F_{1,31}$ = 9.817, P = 0.004), fluid composite score ($F_{1,31}$ = 7.350, P = 0.011), and list-sorting ($F_{1,31}$ = 4.655, P = 0.04) (**Table 4.8**). In the secondary analysis, we observed an effect of impairment status in the same measures (all P<0.05), plus the dimensional card sort ($F_{2,29}$ = 3.453, P = 0.046) (**Table 4.9**). Pairwise tests showed a difference in total composite scores between Asymptomatic and MCI (P = 0.018) and Asymptomatic and MCI+MBI (P = 0.038), but no significant difference between MCI and MCI+MBI (P = 0.635). For the fluid composite score, Asymptomatic was greater than MCI (P = 0.018), but not MCI+MBI (P = 0.060) and MCI was not significantly different from MCI+MBI (P = 0.752). For the list sorting test, MCI was significantly lower than Asymptomatic (P = 0.002), but not MCI+MBI (P = 0.072). Lastly, while the omnibus F-test was significant for the dimension change card sort, no significant differences between the groups were observed in the pairwise tests (all P > 0.05).

Table 4.8: NIH Cognition Toolbox uncorrected standard scores comparing Asymptomatic to Impaired (MCI or MBI). Mean (SD).

	Asymptomatic (n=15)	**Impaired (n=18)**	***F-Statistic***	**p-value**
Crystallized Component Composite Score	112.7 (5.3)	109.2 (5.8)	3.248	0.082
Picture Vocabulary	114.2 (7.0)	109.7 (6.1)	4.134	0.051
Oral Reading Recognition	110.0 (4.0)	108.1 (5.6)	1.165	0.289
Fluid Component Composite Score	95.9 (9.3)	88.5 (8.4)	7.350	**0.011**
List Sorting	105.2 (11.1)	98.2 (9.1)	4.655	**0.039**
Picture Sequence Memory	93.7 (10.2)	88.4 (9.7)	2.346	0.136
Pattern Comparison	89.0 (17.2)	83.1 (14.4)	1.267	0.269
Flanker Inhibitory Control Sort	95.8 (6.5)	92.8 (7.3)	1.595	0.216
Dimensional Change Card	106.2 (6.1)	100.7 (9.1)	4.078	0.052
Total Composite Score	104.4 (7.4)	98.0 (5.9)	9.817	**0.004**

Table 4.9: NIH Cognition Toolbox uncorrected standard scores comparing Asymptomatic, MCI, and MCI+MBI. Mean (SD)

	Asymptomatic (n=15)	**MCI (n=9)**	**MCI+MBI (n=8)**	***F-Statistic***	**p-value**
Crystallized Component Composite Score	112.7 (5.3)	110.8 (5.3)	108.5 (6.0)	1.546	0.231
Picture Vocabulary	114.2 (7.0)	111.3 (5.5)	108.8 (6.8)	1.963	0.159
Oral Reading Recognition	110.0 (4.0)	109.6 (5.4)	107.6 (5.3)	0.665	0.522
Fluid Component Composite Score	95.9 (9.3)	89.1 (6.1)	87.6 (11.3)	3.664	**0.039**
List Sorting	105.2 (11.1)	93.9 (6.6)	102.1 (10.3)	4.797	**0.016**
Picture Sequence Memory	93.7 (10.2)	90.6 (12.4)	85.9 (6.4)	1.588	0.222
Pattern Comparison	89.0 (17.2)	82.9 (13.1)	82.6 (17.4)	0.652	0.529
Flanker Inhibitory Control	95.8 (6.5)	93.6 (5.9)	92.5 (9.3)	0.671	0.519
Dimensional Change Card Sort	106.2 (6.1)	103.9 (4.9)	97.4 (12.1)	3.453	**0.046**
Total Composite Score	104.4 (7.4)	99.2 (4.3)	97.1 (7.6)	4.485	**0.020**

Diffusion Tensor Imaging

We observed a significant effect of impairment status on weighted mean FA between Asymptomatic and Impaired in the left ($F_{1,28}$ = 8.691, P = 0.006) and right uncinate fasciculi ($F_{1,28}$ = 12.337, P = 0.002) (**Figures 4.1** and **4.2**, respectively). No other region was significantly different across the groups (**Table 4.10**). The normal group has greater mean FA in these regions than the impaired group. Secondary analysis comparing the Asymptomatic, MCI, and MCI+MBI groups showed no significant differences between the MCI and MCI-MBI group for either the left (P = 0.810) or right fasciculus (P = 0.829) (**Figures 4.4** and **4.5**, respectively).

In the forceps minor, age was a significant predictor of weighted mean FA ($F_{2,28}$ = 8.605, P = 0.007). To examine the relationship between FA and age in this region, we revised our model to include an interaction term between age and cognitive status; the interaction was not significant (P = 0.742). FA is plotted against age for all subjects in **Figure 4.7**.

Within the left and right uncinate and the forceps minor, we observed no significant differences between the groups in any other tensor parameter, including axial, radial, and mean diffusivity (all P > 0.05).

Neurite Orientation Dispersion and Density Imaging

No difference in OD, ICV, or ISO was observed between the impaired and asymptomatic subjects in the right or left fasciculi (all P> 0.05; **Table 4.11**).

Table 4.10: Weighted fractional anisotropy for each region of interest comparing Asymptomatic to Impaired (MCI or MBI).

Region	**Asymptomatic (n=14)**	**Impaired (n=17)**	**Group**		**Age**	
			F-ratio	**p-value**	***F-ratio***	**p-value**
L Uncinate Fasciculus	0.4334 (0.445)	0.3010 (0.359)	8.691	**0.013**	0.002	0.962
R Uncinate Fasciculus	0.4156 (0.266)	0.3795 (0.290)	12.337	**0.003**	0.024	0.877
L Cingulum (cingulate gyrus end)	0.5843 (0.593)	0.5695 (0.567)	0.482	0.493	0.012	0.889
R Cingulum (cingulate gyrus end)	0.5559 (0.804)	0.5317 (0.502)	1.059	0.312	1.403	0.246
L Cingulum (angular bundle)	0.3287 (0.823)	0.3181 (0.624)	0.161	0.691	0.0003	0.985
R Cingulum (angular bundle)	0.3234 (0.518)	0.3489 (0.469)	2.040	0.164	0.596	0.447
Forceps minor	0.4710 (0.803)	0.4057 (0.599)	2.017	0.116	8.605	**0.007**
Forceps major	0.5973 (0.394)	0.5900 (0.103)	0.061	0.806	0.928	0.344

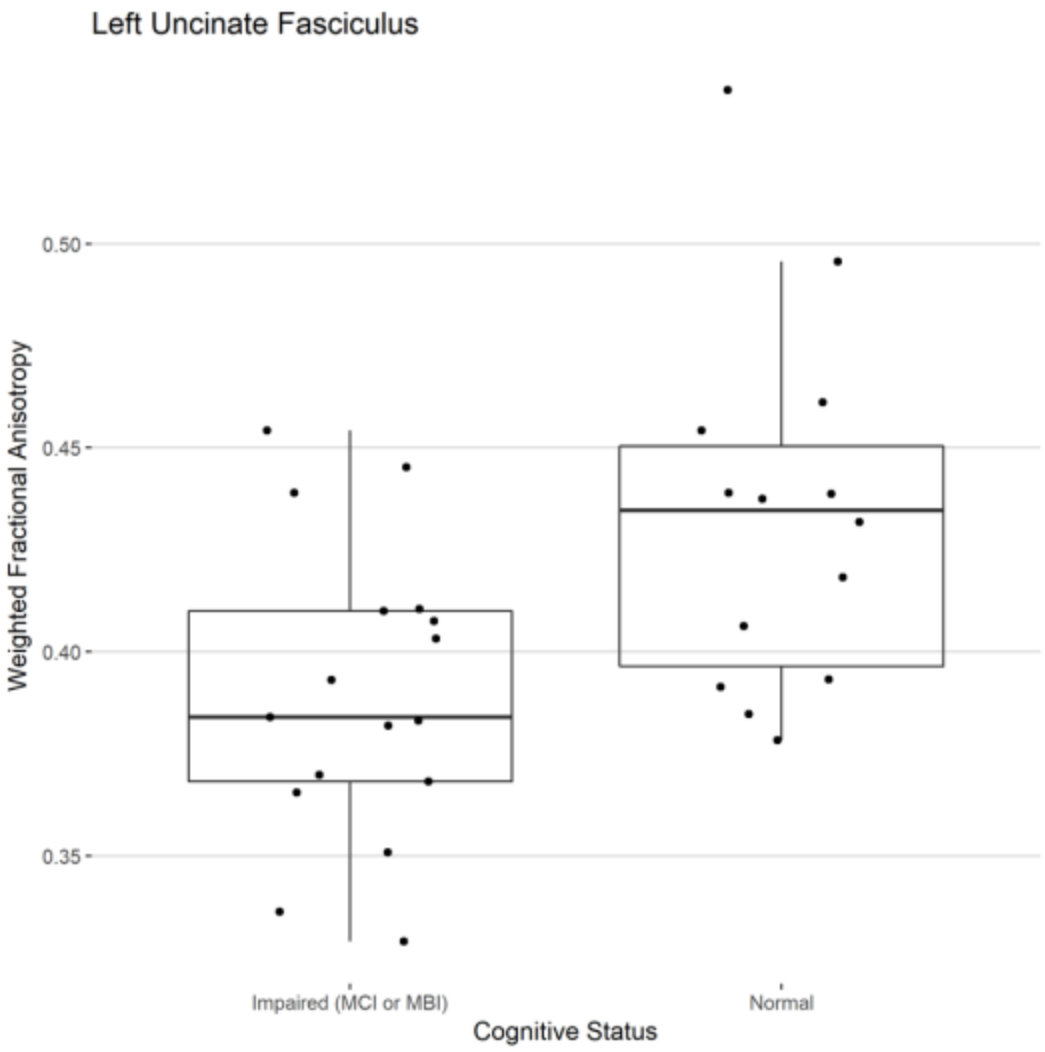


Figure 4.1: Mean weighted fractional anisotropy in the left uncinate fasciculus comparing Asymptomatic and Impaired.

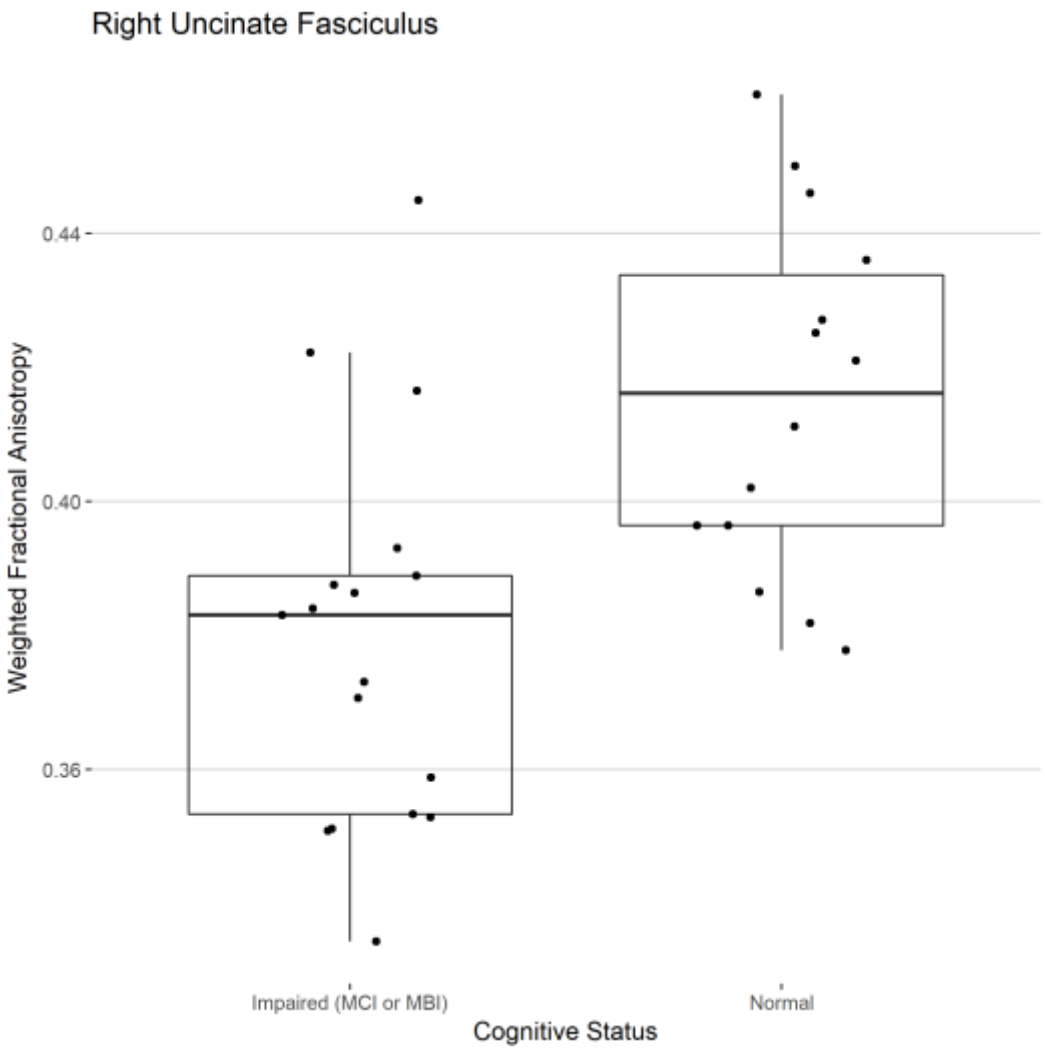


Figure 4.2: Mean weighted fractional anisotropy in the right uncinate fasciculus comparing Asymptomatic and Impaired.

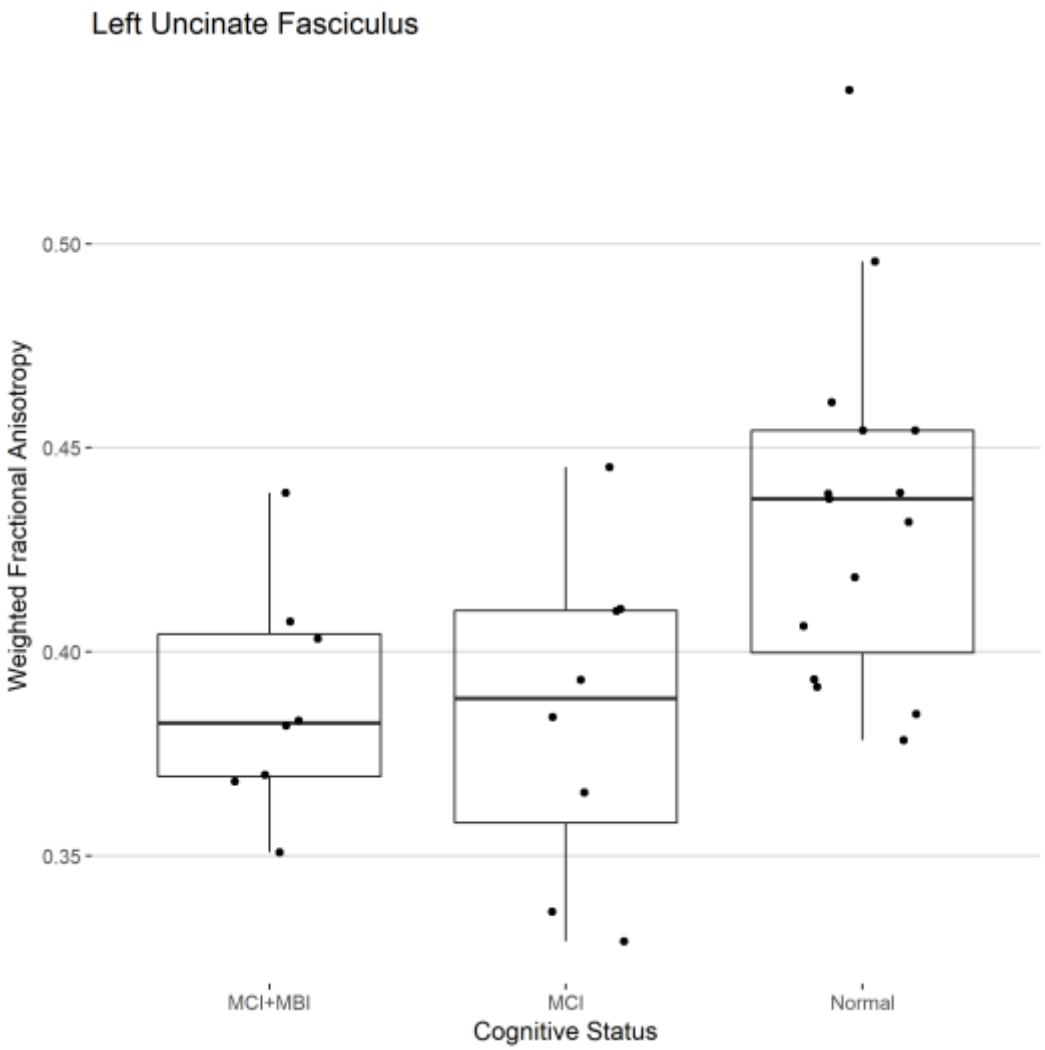


Figure 4.3: Mean weighted fractional anisotropy in the left uncinate fasciculus comparing Unimpaired, MCI, and MCI+MBI.

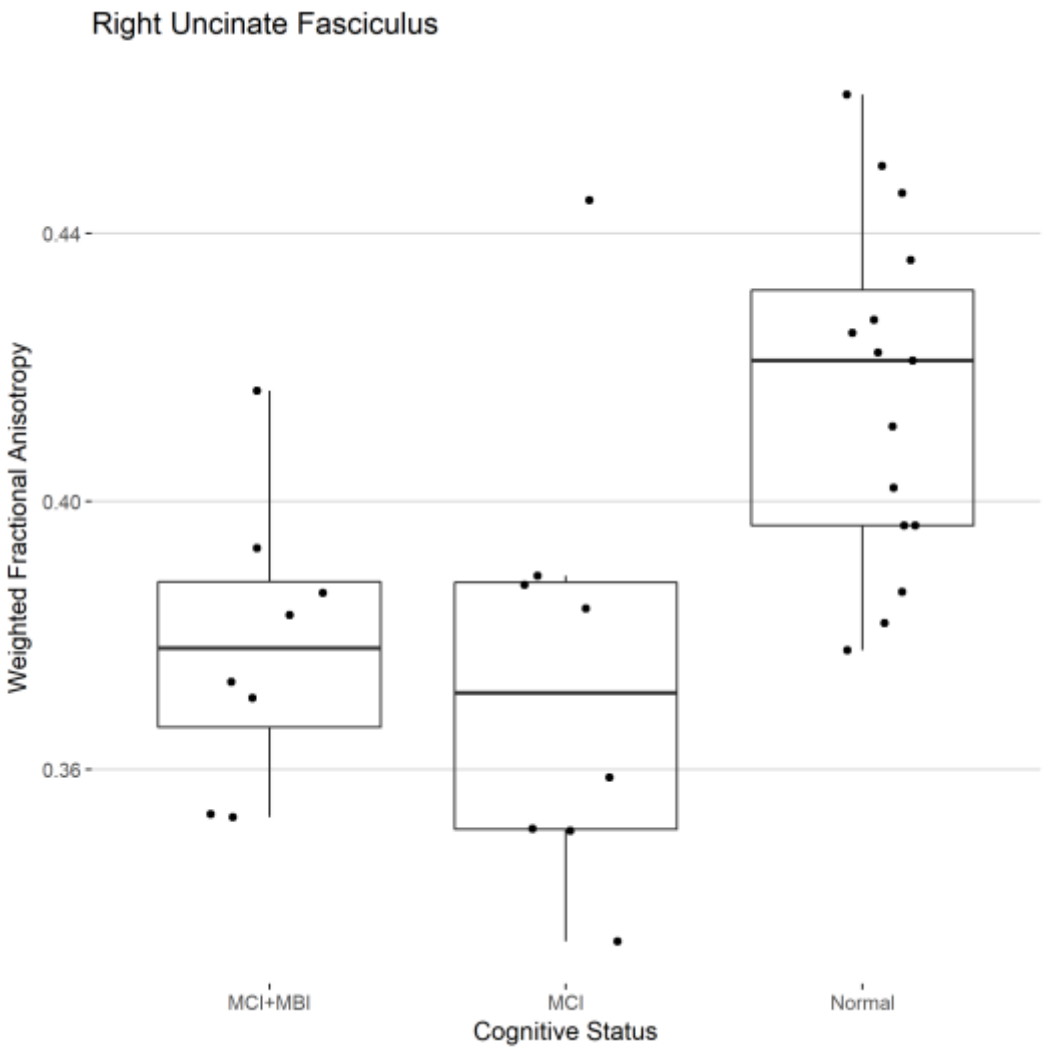


Figure 4.4: Mean weighted fractional anisotropy in the right uncinate fasciculus comparing Asymptomatic, MCI, and MCI+MBI.

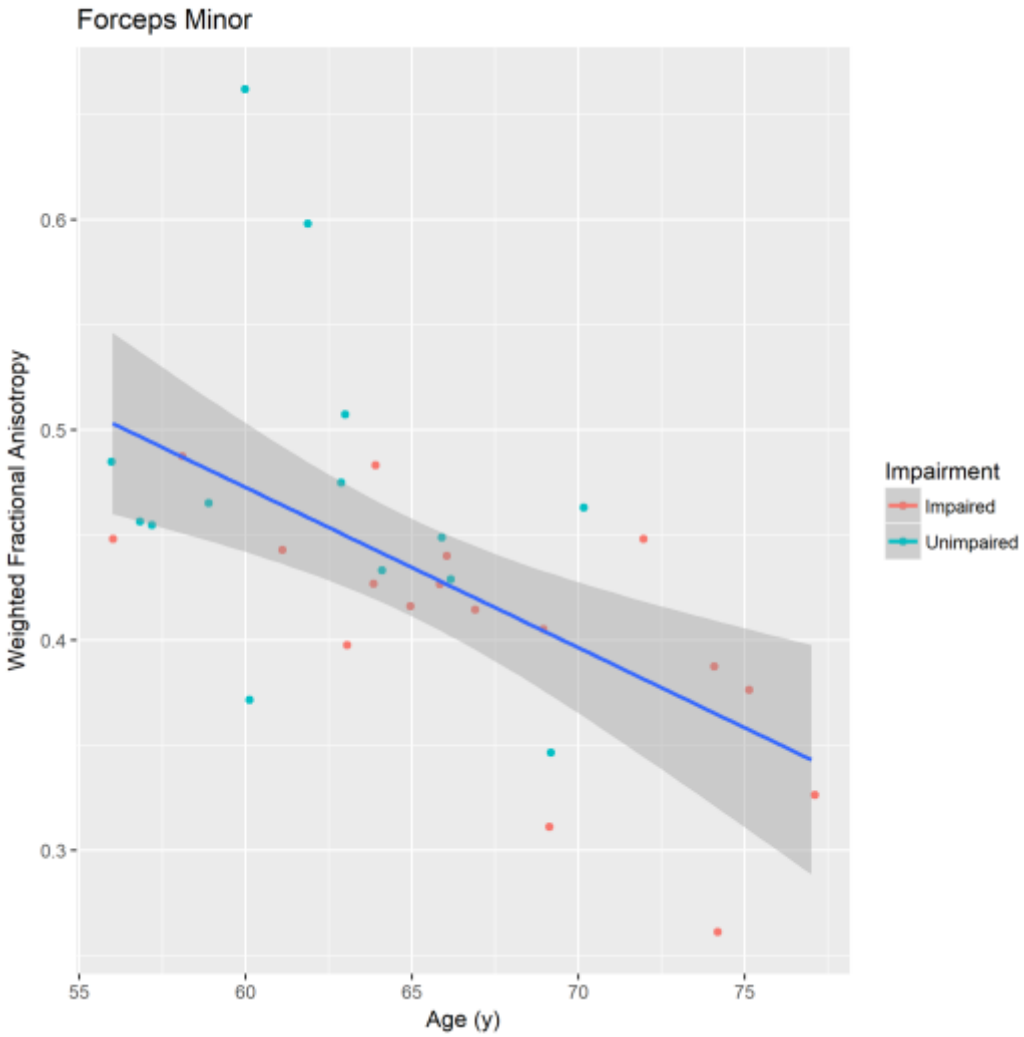


Figure 4.5: Weighted fractional anisotropy in the forceps minor plotted against age with a regression line shown for all subjects with standard error shaded.

Table 4.11: Weighted mean NODDI metrics for each region of interest comparing Asymptomatic to Impaired (MCI or MBI).

	Asymptomatic (n=14)	Impaired (n=17)	Group		Age	
			F-ratio	p-value	*F-ratio*	p-value
Orientation Dispersion						
L Uncinate Fasciculus	0.2805 (0.0841)	0.2838 (0.0411)	0.458	0.504	1.695	0.203
R Uncinate Fasciculus	0.3027 (0.0781)	0.2849 (0.0874)	1.627	0.213	2.910	0.099
Intracellular Volume Fraction						
L Uncinate Fasciculus	0.7097 (0.0806)	0.6620 (0.0764)	1.820	0.188	0.150	0.701
R Uncinate Fasciculus	0.6774 (0.0802)	0.6706 (0.0835)	0.0001	0.993	0.222	0.641
Isotropic Volume Fraction						
L Uncinate Fasciculus	0.1152 (0.0390)	0.1310 (0.0404)	0.835	0.369	0.024	0.879
R Uncinate Fasciculus	0.1070 (0.0359)	0.1304 (0.0500)	1.415	0.244	0.079	0.781

Clinical Correlates to DTI Metrics

We examined correlations between the clinical measures of cognition and neuropsychiatric status and weighted FA in the right and left uncinate fasciculus. For these models, weighted mean FA was predicted by age, impairment status, and the clinical measure of interest, with an interaction term between impairment status and the measure. We limited our search to the measures that were found to be significantly different between the normal and impaired groups. The summary of these models is given in **Table 4.12**. No significant relationship was observed between cognitive scores or psychiatric symptoms and FA in the right or left fasciculi.

Table 4.12: Summary of models assessing clinical correlates to fractional anisotropy (FA).

	L Uncinate Fasc.		R Uncinate Fasc.	
	F-Statistic	**p-value**	***F-Statistic***	**p-value**
Cognitive Paper Assessments				
MMSE	2.095	0.158	1.959	0.172
SDC	3.156	0.086	3.985	0.055
Psychiatric Symptom Surveys				
PHQ-9	0.129	0.722	0.196	0.661
BDI-II	0.527	0.474	0.349	0.559
GAD-7	1.428	0.242	1.213	0.280
BIS (Informant)	0.131	0.720	0.945	0.339
NIH Cognition Toolbox				
Fluid Component Composite Score	3.376	0.076	1.285	0.266
List Sorting	2.304	0.140	2.357	0.136
Dimensional Change Card Sort	0.158	0.477	2.792	0.105
Total Composite Score	1.195	0.283	0.472	0.498

MMSE: Mini Mental State Exam; SDC: Symbol Digit Coding; PHQ-9: patient health questionnaire 9-item scale; BDI-II: Beck Depression Inventory 2nd Edition; GAD-7: Generalized Anxiety Disorder 7-item scale; BIS: Behavioral Inhibition Scale (completed by informant).

Discussion

In this group of former professional football players, we compared cognitive performance and neurobehavioral function between those with MCI (and MBI, in some cases) and those with normal cognition for their age (asymptomatic). We found that those with impairments had lower white matter integrity in the bilateral uncinate fasciculi, suggesting damage to white matter underlies their cognitive or behavioral dysfunction. Interestingly, we did not observe a difference in white matter integrity between those with MCI and those with MCI and MBI, suggesting behavioral impairments do not imply further damage to white matter structures, at least within the frontolimbic network. Our findings suggest an association between white matter damage and the development of early signs of cognitive or behavioral decline in those exposed to repetitive head trauma.

While the clinical presentation of neurodegeneration is expected to be variable, common elements were observed in our study cohort. Of the impaired subjects in our study, the most commonly affected domain of the CDR was memory, with all impaired subjects having at least mild impairment. Judgement and problem-solving abilities were also commonly affected. Approximately half of the impaired subjects also had clinically meaningful behavioral impairment. In this group, the most commonly reported NPIQ symptoms were irritability/lability, aggression/agitation, and apathy/indifference. We sought to examine the neural underpinnings of such impairments in the frontolimbic system.

Our data demonstrate a significant association between cognitive or behavioral impairments and white matter integrity in the bilateral uncinate fasciculi (UF) in former professional football players with history of recurrent concussion. In our study, those with either cognitive impairments or both cognitive and behavioral impairments had lower FA in the UF than the normal subjects. The effect size of these differences was greater on the right than left. However, we did not observe a significant difference in the higher-level NODDI metrics (OD,

ICV, or ISO) between the groups. Given that there was no difference in these other measures of diffusivity, it remains unclear what neuronal process may result in the lower fractional anisotropy of the impaired group without significantly affecting other tensor or NODDI metrics.

We observed lower cognitive performance in the group with either MCI or MCI+MBI compared to the normal group across a number of measures, including MMSE, SDC, and NIH Cognition Toolbox fluid composite and overall scores. Importantly, no differences between the groups were observed in tests of crystallized abilities, which reflect premorbid cognitive function, meaning the difference in fluid abilities is not explained by premorbid cognition. For each cognitive outcome in which group differences were observed, the normal group scored the highest, followed by MCI only, with MCI+MBI group performing the worst. On the NIH Cognition Toolbox, the tests that were associated with impairment status were fluid cognitive abilities, or those that are expected to change with age or neurodegenerative pathology. These primarily include measures of executive function, or the top-down ability to control goal-directed activity. In the development of the Toolbox, its authors chose three subdomains to represent executive function: working memory, set-shifting, and inhibitory control and attention. The three tests that assess these subdomains are list-sorting, dimensional card change, and Flanker inhibitory control task, respectively.[135] Of these, the impaired subjects performed worse on both list-sort and dimensional card change, suggesting impairment in working memory and set-shifting. Conversely, no differences were noted on the Flanker inhibitory control task.

Compared to subjects with only MCI, those with both MCI and MBI had greater symptomatology related to depression, anxiety, and impulsivity, but performed similarly on cognitive measures. Additionally, there was no difference between those with MCI only and those with both MCI and MBI with respect to white matter integrity in the frontolimbic network. This suggests the MBI component of impairment in these subjects may not originate from increased white matter damage in the frontolimbic network. A variety of factors may contribute

to the development of behavioral impairments in those with a history of recurrent head trauma, however, we do not have imaging evidence to explain the presence of neurobehavioral symptoms in our MCI+MBI group.

We did not observe a clinical correlate to the finding of lower FA in the bilateral UF. This lack of clinical correlate is in contrast to previous work. A study of former professional football players found axial diffusivity in the UF were predictive of measures of impulsivity.[89] While impulsivity was significantly greater for the subjects in our study with both MCI and MBI, this impulsivity score did not correlate to FA in the UF. Additionally, a previous study of depressive symptoms in former professional football players reported an association between fractional anisotropy in the forceps minor and symptoms measured by the BDI.[99] We failed to observe such an association in our data, though it is worth noting this previous study did not analyze subjects with MCI, which may account for the discrepancy in our results. We did note a significant relationship between FA and age in the forceps minor, with older subjects tending to have lower FA in this region. However, the linear relationship with age was not affected by impairment status or BDI score.

A limit to the study is that we do not have a healthy control group without exposure to concussive injury. All subjects in our study have a significant history of exposure to repetitive concussive and subconcussive impacts. The effect size of impairment on white matter integrity between our groups may be smaller than the effect size of the impact exposure. Nonetheless, we observed a significant difference in white matter integrity within the bilateral uncinate fasciculus between the impaired and normal subjects. This tract appears to be involved in the early stages of cognitive and behavioral decline in those exposed to repetitive head trauma. More work is needed to determine if this reduction in integrity is a result of increased hyperphosphorylated tau deposition, or if this is related to trauma exposure and predates the development of impairments. Furthermore, it is critical to follow these subjects prospectively to

determine what proportion of subjects may convert to dementia over the next few years. It is possible that the UF provides a marker of either onset or progression of CTE-related neurodegeneration, though more evidence is needed to confirm this hypothesis.

Frontolimbic Neural Recruitment in Former Football Players with Mild Cognitive and Behavioral Impairments

Introduction

Recurrent concussions are associated with increased risk of depression,[1] mild cognitive impairment,[2,3] and the neurodegenerative disease, chronic traumatic encephalopathy (CTE). While the clinical syndrome associated with CTE is not fully understood, current evidence suggests a mix of both cognitive impairments and behavioral disturbances.[129 8] These symptoms are believed to be caused by hyperphosphorylated tau deposition in the frontolimbic neural network, which includes regions in the mediotemporal, temporal, and frontal cortices. More research is needed to better elucidate the character of early cognitive and behavioral decline in former professional football players, a population at especially high risk of CTE.

Functional MRI (fMRI) is a modality that allows for the probing of neural function across a wide variety of pathological and cognitive contexts. Previously, we have used a working memory fMRI paradigm to study former professional football players with variable exposure history, finding that the task was sensitive to differences in neural recruitment patterns between groups across levels of the task. Thus, fMRI may be useful in studying the function of neural networks believed to be involved in CTE-related decline.

In this study, we examine former professional football players with a history of recurrent concussion and variable expression of cognitive and behavioral impairments. Using a working memory N-back task with emotional face distractors, we compare the performance and neural recruitment patterns between those with impairments and those without. The N-back task is known to activate a well-described frontoparietal network comprising six cortical regions: 1) bilateral medial posterior parietal; 2) bilateral premotor; 3) dorsal cingulate; 4) bilateral rostral prefrontal/frontal pole; 5) bilateral dorsolateral prefrontal; and 6) bilateral mid-ventrolateral

prefrontal cortices.[110] In response to facial cues such as the ones serving as our distractors, we expect activation primarily in the bilateral amygdala and visual cortex,[107] with inconsistent activation of the orbitofrontal cortex and inferior temporal regions.

In analyzing the activation patterns of the group in response to the fMRI task, we were primarily interested in whether the presence of distractors would result in aberrant recruitment patterns in the impaired subjects compared to the normal group. Because executive dysfunction has been posited as a feature of CTE, we hypothesized that the impaired group would not be able to efficiently recruit the frontoparietal network when cognitive load is maximal, resulting in a decrease in task performance. Within the normal group, there is an expected decrease in performance at the two-back level; we expect this decrease to be significantly greater for the impaired group. Furthermore, this decrease in performance due to increased cognitive load would be exacerbated when distractor faces placed an attentional demand to interfere with the task.

We hypothesized that damage to frontolimbic connections critical to attentional control when emotionally valent stimuli are presented would result in dedifferentiation of activity during the high cognitive load (2-back) task level. Thus, in the primary analysis, we expected the impaired group would have greater activation of task irrelevant regions within the frontolimbic network compared to controls. In the secondary analysis, we expected the MCI+MBI group to be driving this difference (i.e. they would have greater activation than the MCI and normal group). Based on the previous literature using the task,[136-139] it is likely that we will see differential activity within the frontal lobe between the impaired and normal group. However, as we did not have an a priori hypothesis as to where the cortical activation may be different, we chose to use a whole brain analysis.

By using a working memory task with emotional face distractors, we will engage a broadly distributed frontoparietal network in former professional football players variably

expressing cognitive and behavioral impairments. Converging evidence suggests lower white matter integrity after exposure to repetitive head trauma, however, few studies have examined the functional consequences of damage to white matter. By discerning differences in performance and neural recruitment in response to the fMRI task we hope to identify early functional deficits in those with MCI or MBI. We expect this will be associated with symptoms related to neurobehavioral function, such as impulsivity, depression, or anxiety.

Methods

Participants

From January 2016 to June 2017 we recruited a sample of 35 former National Football League (NFL) players from a registry maintained by the Center for the Study of Retired Athletes (CSRA) at the University of North Carolina at Chapel Hill. Of the approximately 3400 former players in the registry, 1282 report more than two prior concussions and 507 self-reported memory/cognitive problems at some point over the period of 2001-2012. Of these 507, a telephone number was available for 257. Of these, 79 were deceased and 38 had incorrect contact information and were unable to be reached. Of the 150 successfully contacted former athletes, we recruited 36 into the study. All participants gave both verbal and written informed consent in accordance with the requirements of the Institutional Review Board.

Subjects were males between 55 and 80 who played a minimum of three seasons at each of the following levels of football: high school, college, and professional (minimum of nine years of football exposure). Furthermore, they must have reported at least three concussions in their lifetime using a standardized definition of concussion.[114] Exclusion criteria included any diagnosis of dementia including probable Alzheimer's disease, frontotemporal dementia, vascular dementia, or dementia with Lewy bodies; any contraindications for magnetic resonance imaging, including claustrophobia, pacemaker, unsafe metal implants, or weight exceeding 300

pounds; history of moderate or severe traumatic brain injury resulting in hospitalization; severe psychiatric disease such as bipolar or schizophrenia; diagnosis of amyotrophic lateral sclerosis, multiple sclerosis, or history of a major stroke. In this sequence of telephone screeners, if any exclusion criteria were violated, the remaining instruments were not administered. Once inclusion/exclusion criteria were met, enrolled subjects were brought to the University of North Carolina at Chapel Hill for assessment.

One subject in the MCI group could not complete any imaging sequence due to claustrophobia. One subject in the MCI+MBI group did not complete the fMRI sequence because of scanner hardware problems. Another subject in the asymptomatic group was unwilling to complete the fMRI sequence because the scanning session ran much longer than usual, again due to scanner hardware issues. Data for one subject in the asymptomatic group were unable to be processed using the FreeSurfer processing stream because of significant global atrophy. This subject's imaging data were discarded as the analysis of diffusion-weighted and functional images depends on the FreeSurfer surface reconstruction and the mapping of anatomical priors onto the processed T1 image.

Classification of Mild Cognitive and Behavioral Impairment

Impairment status was determined during the in-person visit. Subjects were classified as having mild cognitive impairment (MCI), mild behavioral impairment (MBI), or both. To assess MCI, a trained research assistant administered the Clinical Dementia Rating (CDR). Subjects with a CDR score of 0.5 were classified as MCI. Two subjects scored a 1 on the CDR and were excluded from the analyses as this reflects greater than mild impairment. We classified MBI using the neuropsychiatric inventory questionnaire (NPIQ); subjects with two or more symptoms of greater than mild severity and which cause more than mild distress in the informant were classified as having MBI. Those in the asymptomatic group did not meet criteria for either MCI

or MBI. In total, 15 subjects were impaired (7 with MCI, 8 with MCI+MBI) and 13 subjects did not meet criteria for MCI or MBI, and thus, were classified as asymptomatic.

Image Acquisition

A sagittal T1-weighted magnetization prepared rapid gradient echo (MPRAGE) anatomical sequence was acquired with a voxel size of 1x1x1mm^3 over 192 slices with TR/TE = 1900ms/2.26ms. Additionally, a T2-weighted sequence was acquired with the same voxel size and number of slices with TR/TE=3200ms/402ms. Functional MR data were acquired in one run of approximately 15 minutes on a Siemens Biograph mMR with voxel size 3mm^3 over 47 slices with TR/TE=3000/25ms. Between each slice, a 0.5mm skip was added to ensure full coverage of the cerebrum. Prior to entering the scanner, subjects were familiarized with the in-scanner working memory task. This familiarization session entailed an overview of the n-back which included practice runs for each level (0-, 1-, and 2-back) without visual distractors. Subjects were given feedback on their performance until they were able to complete each level with 100% accuracy.

Working Memory N-back with Emotional Distractors

The task was presented in a blocked design with a pseudorandom sequence of letters flanked by two emotionally expressive faces. In the most basic condition (0-back), subjects were told to press a button when they saw a specified letter (“M”); in the low cognitive load condition (1-back), subjects were told to press a button when they saw a letter repeated back-to-back (e.g., “A”-“A”); at the higher attentional load condition (2-back), they are asked to press a button when a letter is the same as the one presented two stimuli earlier (e.g. “A”-“B”-“A”). For each attentional load level, there are two distracter conditions, neutral faces and fearful faces, and a condition without facial distractors (Blank). Each trial consists of a letter presented in the middle

of the screen with either no distracter faces, or two identical faces flanking either side of the letter. With each stimulus presentation, a new face was presented. Subjects completed 1 run of 18 blocks, with 12 trials in each block (total duration of 15 minutes). Stimuli were presented for 500ms with the inter trial interval consisting of a fixation cross. The inter-trial interval was jittered with a mean duration of 3500ms. At the beginning of each run, instructions were presented to remind the subject how to complete the task.

Image Preprocessing and Analysis

Anatomical T1 images were processed using the FreeSurfer *recon-all* command, with the T2-weighted images used to improve definition of the pial surface. The FreeSurfer segmentation and parcellation processing stream includes the pre-processing steps of removing non-brain voxels (skull-stripping), registration of the T1- and T2-weighted images, cortical surface reconstruction, cortical and subcortical segmentation, and volume, cortical thickness, and surface area estimation. The FreeSurfer image analysis suite is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/).

Functional MRI data were analyzed using FreeSurfer's Functional Analysis Stream (FS-FAST) 5.1. The FS-FAST analysis is a surface-based analysis, which improves spatial localization and allows for better visualization of activations on the cortex.[126,127] First, the middle volume of the run was used as the target for registration of all other run volumes. This middle volume was also used for registration to the anatomical T1 scan using FreeSurfer's *bbregister*. Next, the images were intensity normalized to ensure that the scale of voxel intensities over time is equal. The normalized images are then registered to the T1 anatomical scans using a 6 degree-of-freedom registration to the white-grey matter surface. The motion parameters files were used later in the analysis as nuisance regressors to account for inter-subject variability in motion during the task, which may be associated with the cognitive state of the subjects. The

motion-corrected volumes were slice-time corrected to account for the interleaved acquisition of image slices. The pre-processed data are then resampled to three common spaces: the left and right hemispheres of *fsaverage*, the study-specific common space to which the surface data from the FreeSurfer reconstructed anatomical surfaces are resampled, and the MNI 305 template (used for subcortical analyses). Finally, a 6mm full-width, half-maximum smoothing kernel is applied to the data.

FreeSurfer's general linear model tool, *mri_glmfit,* was used to statistically analyze the fMRI BOLD data. A first-level analysis was performed across all subjects (one-sample group mean) with exhaustive contrasts of both the task levels and the distractor conditions. This involved separate contrasts of the n-back levels (e.g. 1-back>0-back, 2-back>1-back, 2>0-back) at each distractor condition and then collapsing across distractor conditions. The same method was employed for the distractor conditions (e.g. neutral faces > no distractor, fearful faces > neutral faces, and fearful faces > no distractor) at each level of the n-back and then collapsing across all n-back levels. In total, 24 first-level contrasts were used. Next, the group-level contrasts compared the asymptomatic group to the impaired group. Finally, a secondary analysis was performed after sub-stratifying the impaired group to those with behavioral impairments and those without and comparing across the three groups (e.g. Asymptomatic > MCI+MBI, Asymptomatic > MCI, MCI > MCI+MBI). In this secondary analysis, an omnibus F-test was used to first determine any significant differences in BOLD signal change. In the case of a significant F-test, the individual contrasts between the groups were compared. Motion parameters and age were entered as nuisance regressors into the group-level analyses.

Comparison for multiple corrections was performed on the statistical maps resulting from the first- and group-level analyses using the FreeSurfer tool, *mri_glmfit-sim*. This correction first sets a voxel-wise alpha of 0.001 for cluster formation (false discovery rate correction) and a cluster-wise correction with an alpha of 0.05.

Emotional faces N-back task performance

In analyzing task accuracy, our primary interest was whether the presence of distractors would differentially affect accuracy across n-back levels for the Asymptomatic and Impaired groups. When the cognitive load of the task is increased in the 2-back level, we expected the added attentional demands of the distractor conditions would affect the impaired group more than the controls. Accordingly, we hypothesized that the presence of both neutral and fearful faces would lead to worse performance at the 2-back level in those with impairments compared to the asymptomatic subjects. Based on previous work using this paradigm, we expected the fearful faces would affect performance more than the neutral faces at the two-back level. In the secondary analysis, we expected that those with MCI and MBI would drive this difference between the asymptomatic and impaired subjects as this group is expressing symptoms related to frontolimbic dysfunction. When distractors were not present, we expected that the impaired group would have similar performance to the asymptomatic group at the 0- and 1-back levels, but worse performance at the 2-back level.

To address these hypotheses, we first analyzed task accuracy across n-back levels at each distractor condition. In the primary analysis, we used an analysis of variance with accuracy being predicted by impairment status and distractor condition, with an interaction term included in the model. We then analyzed the effect of n-back level and impairment status at each distractor condition using separate ANOVAs. For the secondary analysis, we repeated these models, substratifying the impaired group to MCI and MCI+MBI. Where significant interaction effects were observed, we examined these interactions using Wilcoxon rank sum tests (a non-parametric test was chosen to account for the small and unbalanced sample sizes).

We used a non-parametric analysis, contrasting each level of the task. The 0-back level served as the baseline for activity at the 1- and 2-back levels. We also contrasted the 2-back to the 1-back to determine the effect of added cognitive load. The first analysis of activation

collapsed across groups to determine the common areas of activation. First, we restricted this to the Blank condition to see the common network activated specifically in response to the n-back task

Results

Task accuracy summary statistics by N-back level and distractor condition for the primary analysis are provided in **Table 4.13** with the secondary analysis in **Table 4.14**. In the first pass analysis of task performance, we collapsed across group assignment to determine the effects of N-back level and distractor condition on overall accuracy for the entire sample. In this model, a significant main effect of n-back level was observed ($F_{2,216}$ = 18.879, P <0.001), as well as an interaction between n-back level and distractor condition ($F_{2,216}$ = 2.544, P = 0.041; **Table 4.15**). In a revised model that included impairment status, a main effect of impairment was observed ($F_{1,26}$ = 5.325, P = 0.029; **Table 4.16**); no interaction effects between impairment and n-back level or impairment and distractor condition were significant across all n-back conditions.

Table 4.13: N-back task performance by n-back level and distractor condition comparing Asymptomatic and Impaired (MCI or MBI). Mean (SD).

N-back level	Asymptomatic (n=13)			Impaired (n=15)		
	Blank	**Neutral**	**Fearful**	***Blank***	***Neutral***	**Fearful**
0-back	98.1 (3.7)	99.04 (3.5)	98.72 (3.1)	96.7 (8.8)	98.33 (3.1)	98.06 (3.1)
1-back	99.36 (1.6)	99.36 (1.6)	99.68 (1.2)	98.89 (1.9)	96.11 (7.5)	96.39 (5.6)
2-back	97.44 (1.6)	93.91 (7.5)	97.12 (3.9)	94.44 (7.3)	92.78 (6.2)	91.67 (7.2)

Table 4.14: N-back task performance by n-back level and distractor condition comparing Asymptomatic, MCI, and MCI+MBI. Mean (SD).

N-back level	Asymptomatic (n=13)			MCI (n=8)			MCI+MBI (n=7)		
	Blank	**Neutral**	**Fearful**	***Blank***	***Neutral***	**Fearful**	***Blank***	***Neutral***	**Fearful**
0-back	98.1 (3.7)	99.0 (3.5)	98.7 (3.1)	100.0 (0.0)	99.0 (2.9)	99.0 (1.9)	92.9 (12.2)	97.62 (3.3)	97.02 (4.0)
1-back	99.4 (1.6)	99.4 (1.6)	99.7 (1.2)	99.0 (1.9)	98.4 (3.1)	96.9 (7.3)	98.81 (2.0)	93.45 (10.2)	95.83 (3.40)
2-back	97.4 (3.2)	93.9 (7.5)	97.1 (3.9)	95.8 (7.0)	94.3 (4.4)	95.3 (5.2)	92.86 (7.9)	91.07 (7.8)	87.50 (7.2)

Table 4.15: Analysis of Variance Type III sums of squares with Satterthwaite approximation for degrees of freedom of N-back task performance across entire sample.

Independent Variable	Sum Sq.	Mean Sq.	Num. df	Denom. df	F-statistic	P-value
N-back level	70.90	360.45	2	216	18.879	<0.001
Distractor Condition	14.88	7.44	2	216	0.390	0.678
Interaction	194.28	48.57	4	216	2.544	0.041

Table 4.16: Analysis of Variance of N-back task performance including Impairment status and interactions. Type III sums of squares with Satterthwaite approximation for degrees of freedom.

Independent Variable	Sum Sq.	Mean Sq.	Num. df	Denom. df	F-statistic	P-value
N-back level	235.34	117.67	2	208	6.186	0.002
Distractor Condition	27.01	13.50	2	208	0.710	0.492
Impairment	101.30	101.30	1	26	5.325	0.029
N-back*Distractor	75.38	18.845	4	208	0.991	0.414
N-back*Impairment	23.96	11.98	2	208	0.630	0.534
Distractor*Impairment	21.64	10.82	2	208	0.569	0.567
N-back*Distractor*Impairment	121.58	30.396	4	208	1.598	0.176

To visualize between group effects across n-back level at each distractor condition, accuracy across n-back levels was plotted separately for each distractor condition (primary analysis in **Figure 4.7**, secondary analysis in **Figure 4.8**). To visualize between group differences across distractor condition at each n-back level, accuracy was plotted across distractor condition for each n-back level in **Figure 4.9** and **Figure 4.10**).

In the blank condition (no distractors), no significant effects of n-back level ($F_{2,52}$ = 0.478, P = 0.622) or impairment ($F_{1,26}$ = 1.697, P = 0.204) on accuracy were observed. In the neutral faces distractor condition, a significant effect of n-back level was observed ($F_{2,52}$ = 4.801, P = 0.012) with no significant effect of impairment group ($F_{1,26}$ = 1.581, P = 0.220), and no interaction between n-back and impairment group ($F_{2,52}$ = 0.510, P = 0.603). In the secondary analysis, including MCI+MBI did not result in a significant interaction term ($F_{4,50}$ = 0.490, P = 0.742). Collapsing across impairment groups, performance on the 2-back level was significantly lower than either the 0-back (P < 0.001) or 1-back levels (P = 0.013).

In the fearful faces condition, a significant effect of impairment status on accuracy was observed ($F_{1,26}$ = 6.303, P = 0.019) in the primary analysis. No effect of n-back level ($F_{2,52}$ = 1.446, P = 0.245) and no significant interaction was observed ($F_{2,52}$ = 2.653, P = 0.080). Probing further into the effect of impairment, when MCI+MBI was added as a level to the analysis, a significant interaction effect of impairment status and n-back level was observed ($F_{4,50}$ = 3.313, P = 0.0175). Pairwise tests at each level of the n-back showed no significant difference between the groups at the 0-back level (all Ps > 0.05, corrected). At the 1-back level, the MCI+MBI group had significantly lower accuracy than the asymptomatic group (P = 0.023), but not the MCI group (P = 0.162). At the 2-back level, the MCI+MBI group had significantly lower accuracy than the asymptomatic (P = 0.011) and MCI (P=0.037) groups. No difference between the MCI and asymptomatic groups was observed at any level (all Ps >0.05, corrected).

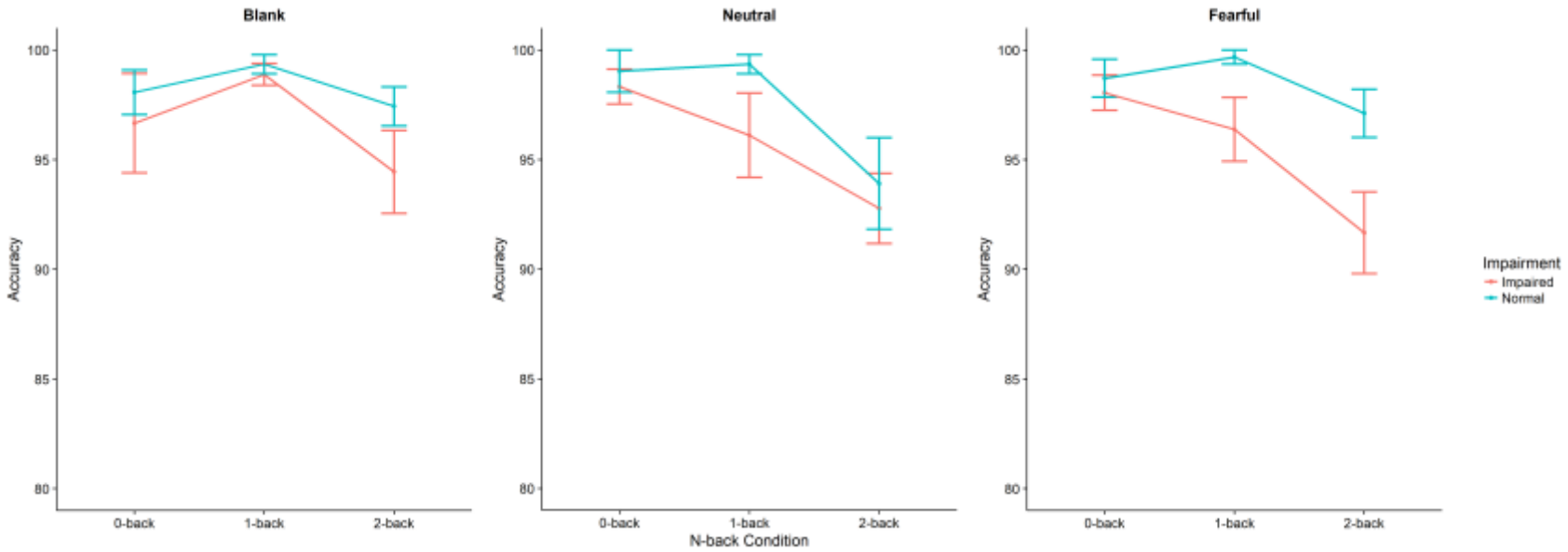


Figure 4.7: Mean task accuracy across n-back levels plotted separately for each distractor condition comparing Normal(Asymptomatic) and Impaired (MCI or MBI). Standard error bars shown.

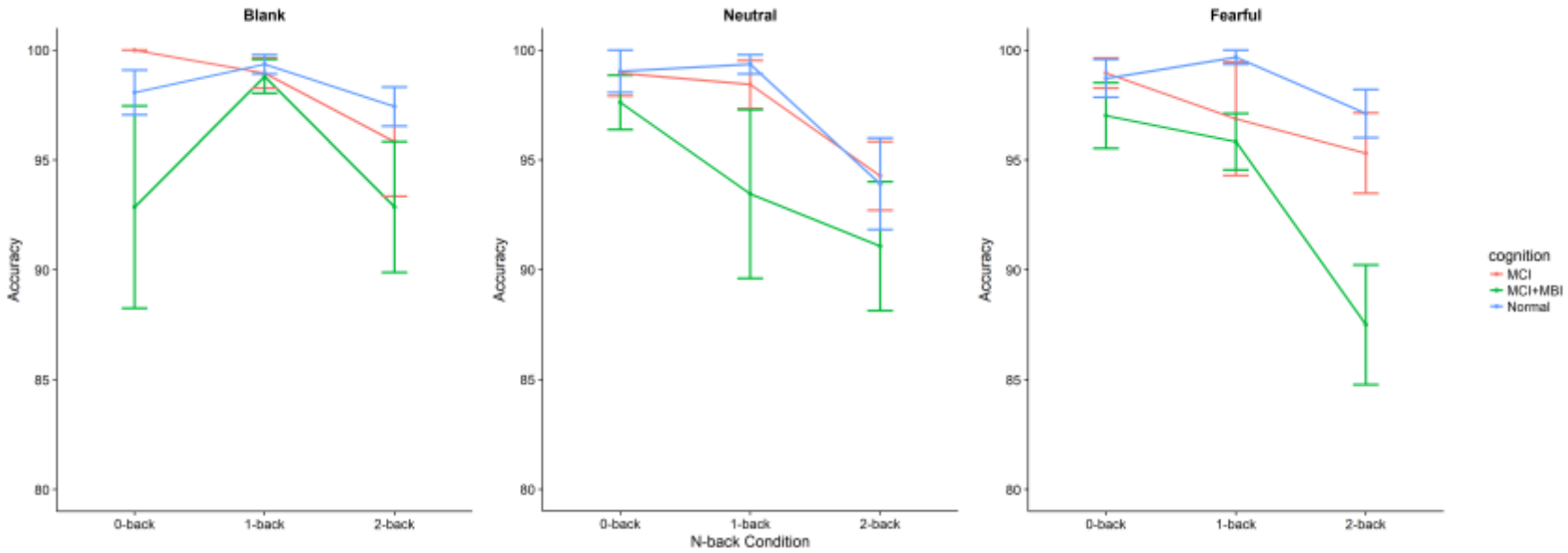


Figure 4.8: Mean task accuracy across n-back levels plotted separately for each distractor condition comparing Normal(Asymptomatic), MCI and MCI+MBI. Standard error bars shown. Note: all subjects in the MCI group completed the 0-back, blank condition with 100% accuracy.

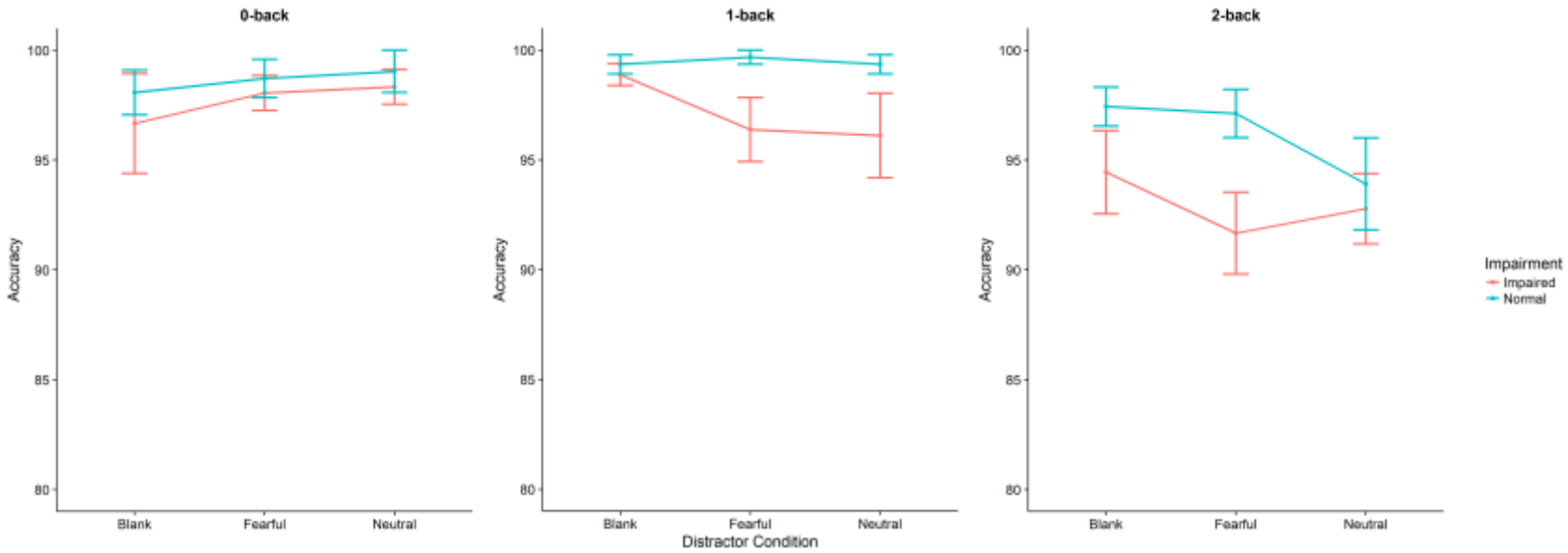


Figure 4.9: Mean task accuracy across distractor conditions is plotted separately for each n-back level comparing Normal(Asymptomatic) and Impaired (MCI or MBI). Standard error bars shown.

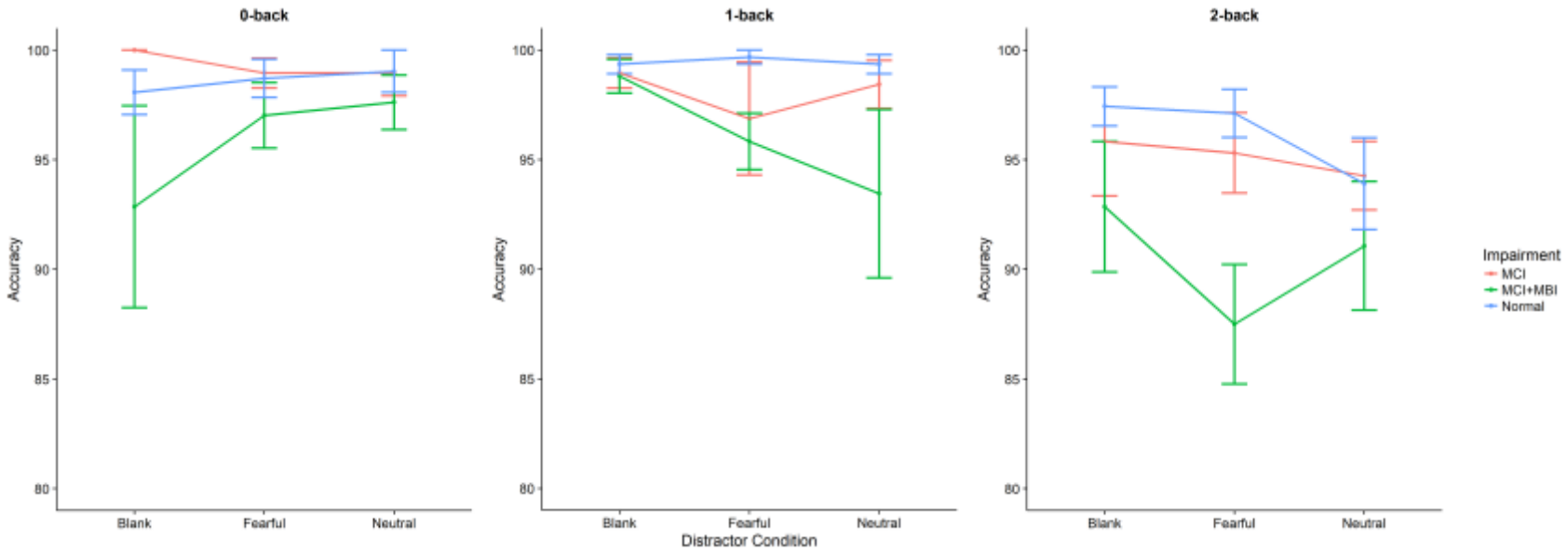


Figure 4.10: Mean task accuracy across distractor conditions is plotted separately for each n-back level comparing Normal(Asymptomatic), MCI and MCI+MBI. Standard error bars shown. Note: all subjects in the MCI group completed the 0-back, blank condition with 100% accuracy.

Neural recruitment during the fMRI task

First-level analysis of activation in response to the task

In the first group level analysis of the BOLD fMRI data, we restricted our analysis to the blank condition and collapsed across impairment status to determine the regions that are commonly activated in response to the N-back task alone. We found broad clusters of activation in the frontoparietal network, including regions in the medial lateral and dorsal lateral prefrontal cortex, which increased with cognitive load (**Figure 4.11)**.

Between Group Analysis – Effects of cognitive load in the blank condition

We observed no significant differences between the groups in the 1-back greater than 0-back and 2-back greater than 0-back contrast. In the 2-back greater than 1-back contrast, a cluster in the precuneus was significantly different between the groups using an omnibus F-test (cluster-wise $P<0.05$). When plotting the BOLD-percent signal change (BOLD-PSC), we observed the MCI+MBI group was driving this difference with activation in this region compared to the deactivation seen in the MCI and asymptomatic groups (**Figure 4.12**)**.** Post-hoc t-tests showed the MCI+MBI group has significantly greater BOLD-PSC than either the MCI ($P = 0.001$) or asymptomatic ($P < 0.001$) groups.

Between Group Analysis – Effects of distractor condition across N-back levels

Collapsing across N-back levels, we observed no significant difference between the groups when comparing the neutral faces distractor condition to the blank condition or the fearful faces to neutral faces. When restricting to the 2-back level, three significant vertex clusters were identified as significantly different between the groups in the comparison of fearful faces to the blank condition (cluster-wise $P < 0.05$). Two clusters lie within the right superior frontal region, the other in the left temporal pole. In plotting the mean BOLD-PSC from the

voxels within the clusters, we observed the superior frontal region was activated to a greater extent by MCI+MBI group than either the MCI (P = 0.002) or asymptomatic groups (P = 0.002) (**Figure 4.13**). Conversely, the temporal pole was deactivated more in the MCI+MBI group compared the MCI (P = 0.021) and asymptomatic groups (P < 0.001). No differences were observed between the MCI and asymptomatic groups for either region (Ps > 0.05). No other group differences were observed in neural recruitment in response to the task.

Clinical Correlates of BOLD-PSC

We examined correlates of behavioral function to BOLD-PSC in the superior frontal and temporal poles. We observed a significant relationship between BOLD-PSC in the superior frontal region and total beck depression inventory II (BDI-II) score ($F_{1,26}$ = 5.071, P = 0.033; **Figure 4.14**). Subjects with greater BDI-II scores tended to have greater activity in the superior frontal region. We observed the opposite relationship in the temporal pole, where greater BDI scores tended to correlated with lower BOLD PSC in the temporal pole ($F_{1,26}$ = 6.253 ; P = 0.019; **Figure 4.15**)

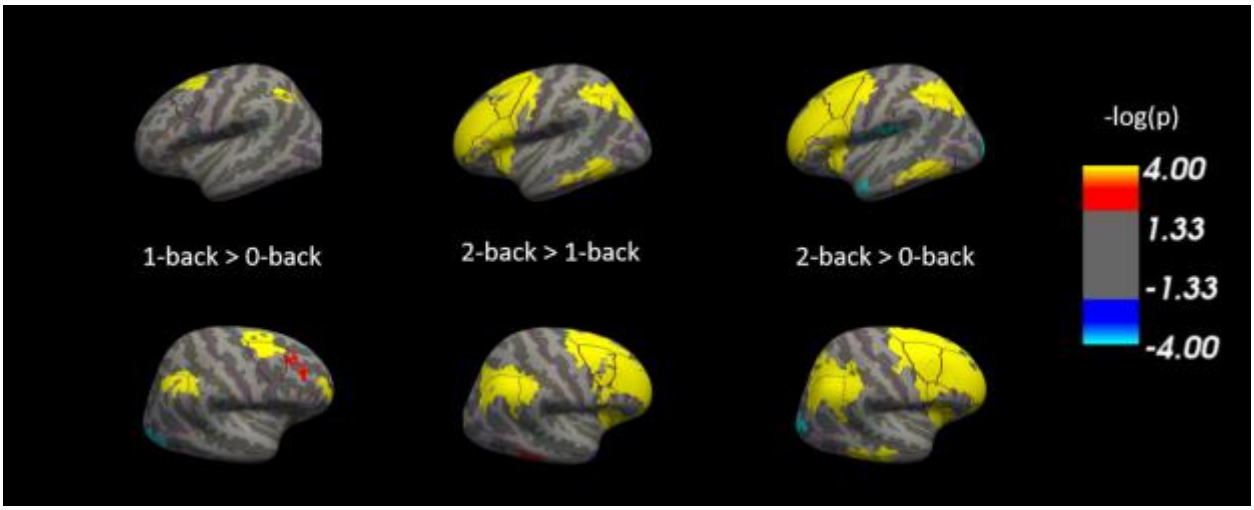


Figure 4.11: Corrected statistical parametric maps showing clusters that are significantly activated (red-yellow) or deactivated (blue-light blue) in response to the task for the cohort as a whole. Cluster-wise $P < 0.05$.

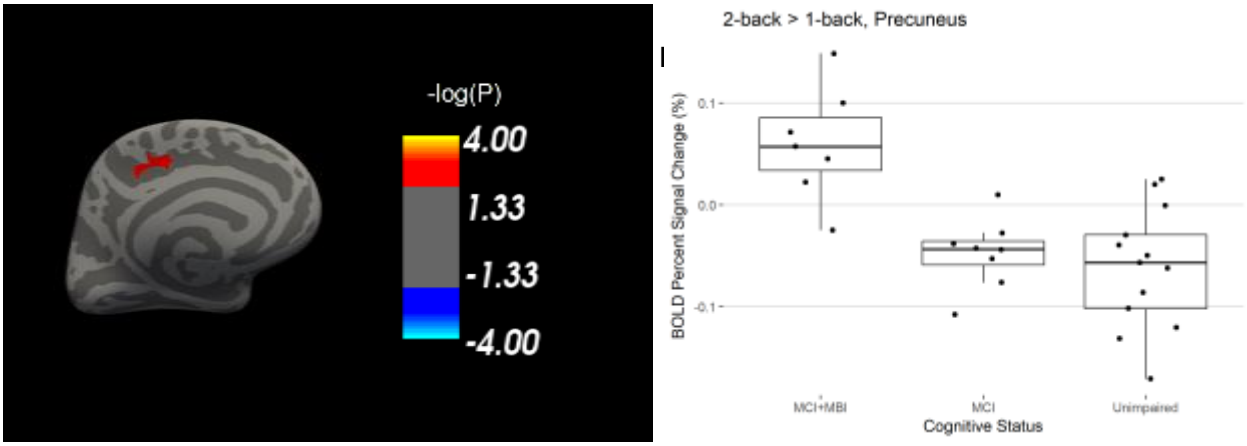


Figure 4.12: A) Cluster of voxels in the left precuneus with significantly different activation between the groups (cluster-wise P<0.05). B) Mean percent signal change was extracted from voxels within the cluster and plotted by group.

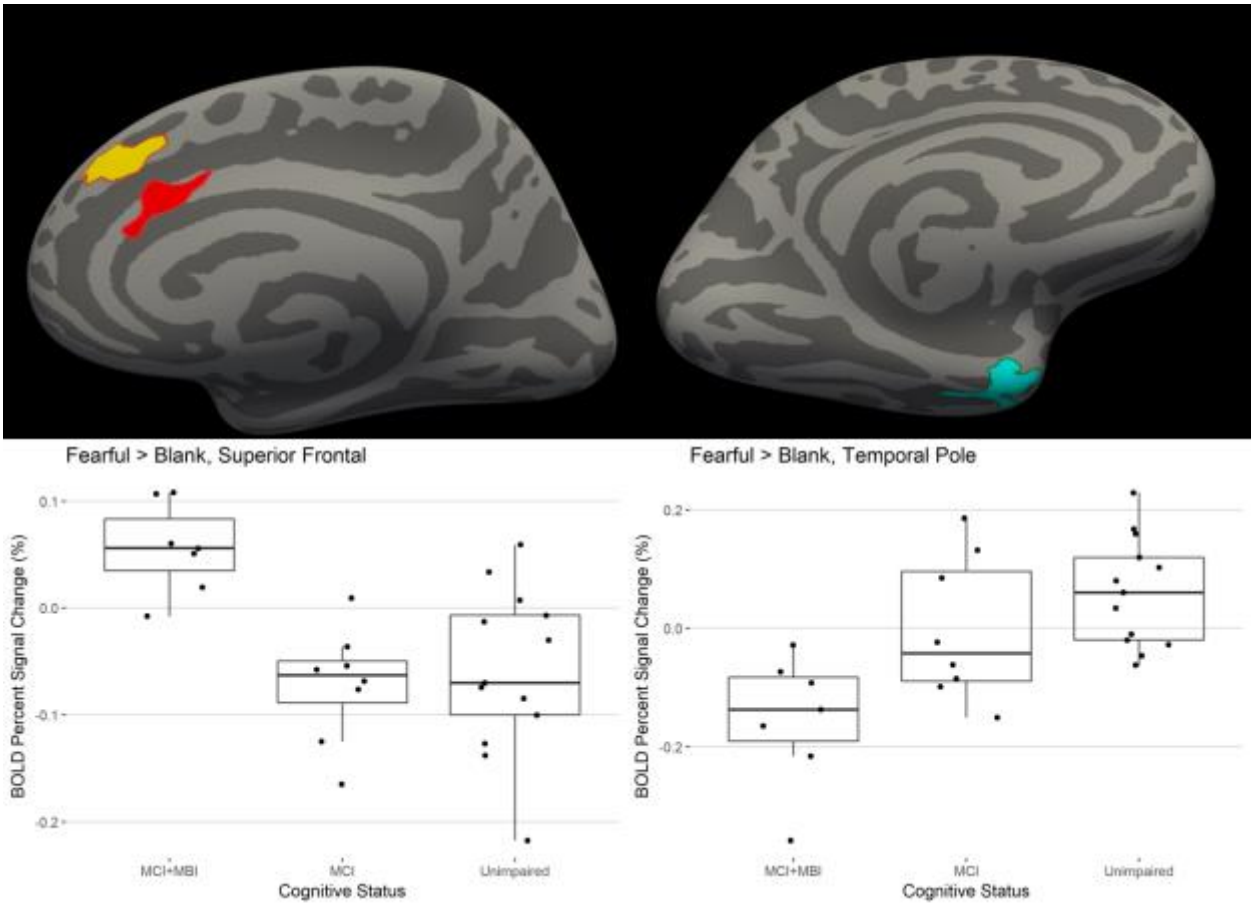


Figure 4.13: A) Significant clusters in the 2-back condition, Fearful > Blank contrast omnibus F-test of difference between impairment groups. B) Mean BOLD-PSC from voxels within the two clusters in the superior frontal lobe, and C) Mean BOLD-PSC from voxels in the temporal pole cluster.

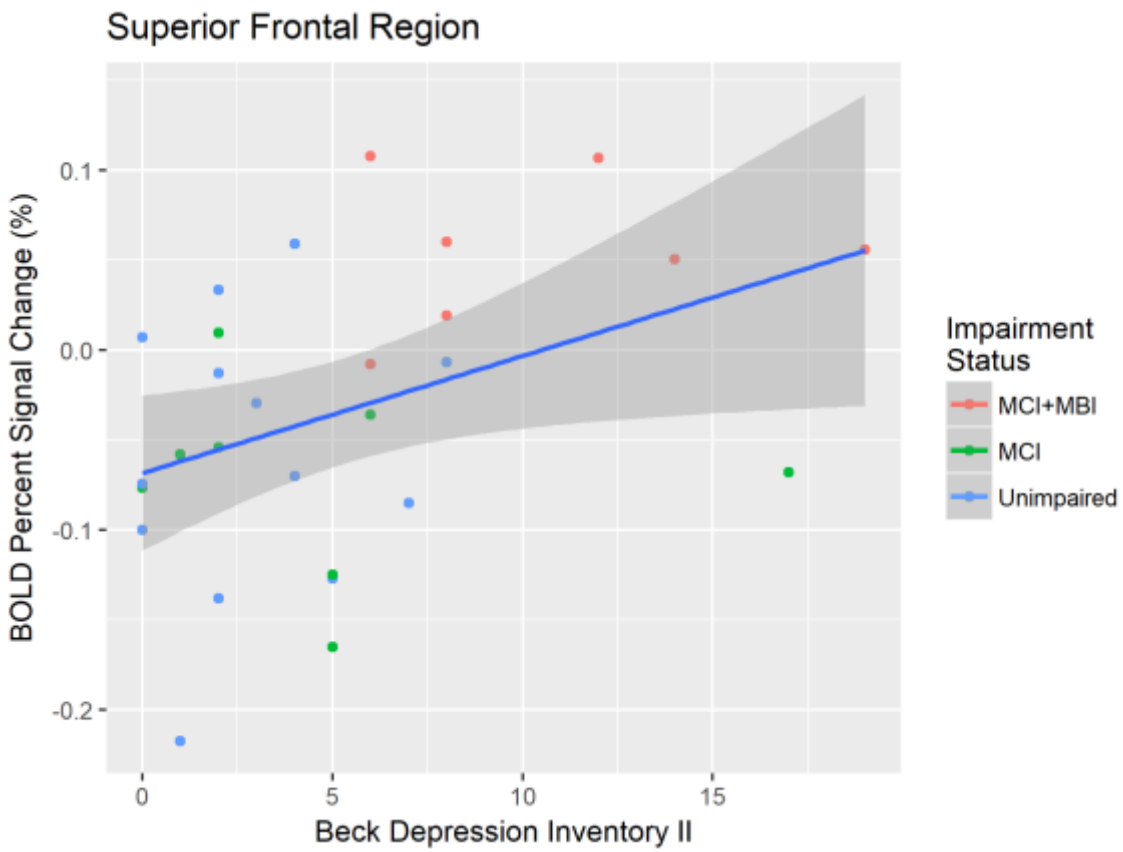


Figure 4.14: BOLD-PSC from the voxel clusters in the superior frontal region plotted against total Beck depression inventory score.

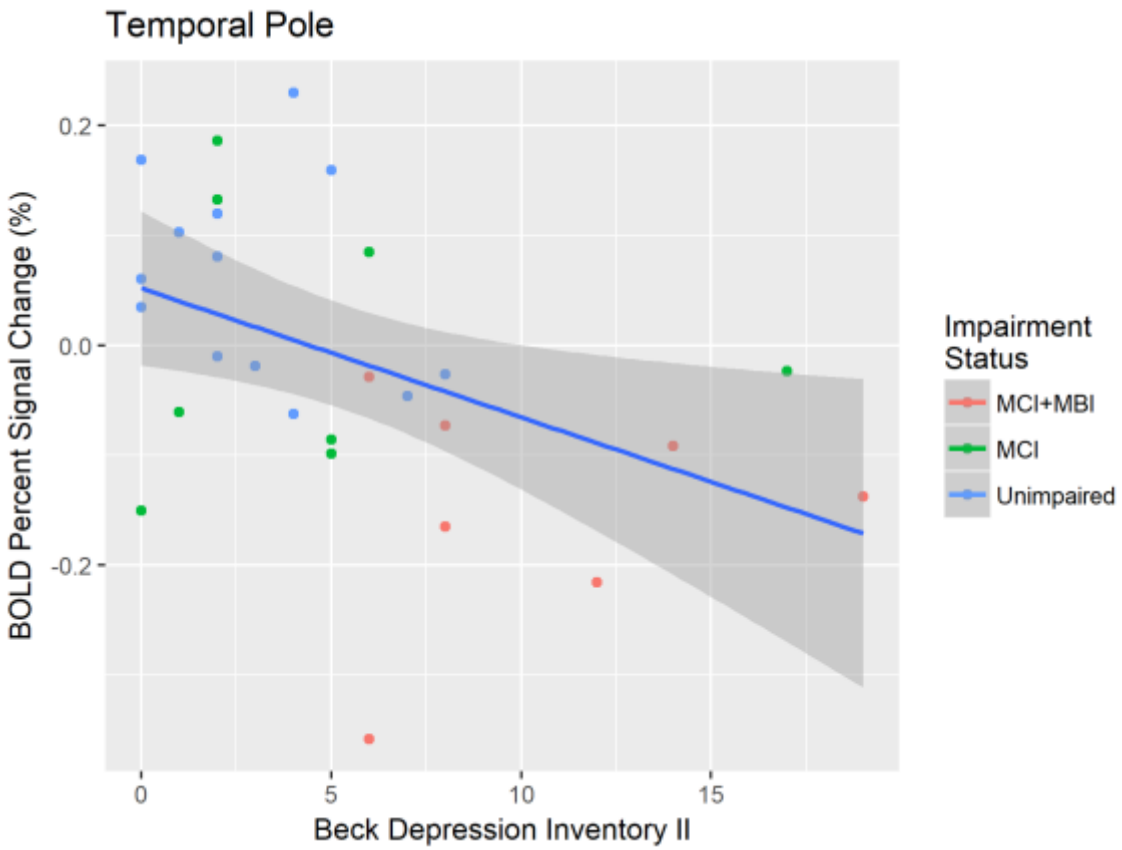


Figure 4.15: BOLD-PSC from the voxel cluster in the temporal pole plotted against total Beck depression inventory score.

Discussion

In our analysis of working memory N-back task performance, we observed a significant effect of impairment status on accuracy, with the impaired group performing worse than the asymptomatic group. This difference was primarily driven by the MCI+MBI group having lower performance at the 2-back level than the asymptomatic and MCI groups in the presence of fearful face distractors. This suggests the addition of MBI on top of MCI is associated with a reduced ability to maintain attentional control when engaging in a working memory task, particularly when greater attention is required to successfully complete the task.

In analyzing the neural recruitment in response to the task, the MCI+MBI group over-recruited the precuneus relative to the MCI and asymptomatic groups when comparing across cognitive load in the absence of distractors. When distractors were present, the MCI+MBI subjects had altered activation in the superior frontal and temporal pole regions. This suggests that when the cognitive load of the task is increased, the MCI+MBI group requires more resources in order to complete the task at an equivalent level as the MCI and asymptomatic groups. When distractors are then added to the high-load task, the demand on cognitive resources is further increased and becomes too great for the MCI+MBI group to compensate.

The MCI+MBI group's inability to efficiently recruit resources for the task may be the result of dysconnectivity between frontal and paralimbic regions, as evidenced by over-activation of the superior frontal lobe temporal pole and under-activation of the temporal pole when fearful faces are added to the 2-back condition. The temporal pole underlies emotional processing and facial recognition.[140] It is typically involved in temporal variant frontotemporal lobar degeneration, where atrophy in the temporal pole is associated with personality changes and reduced social functioning and emotional regulation, as well as semantic memory impairment. We observed a relationship between depression symptomatology and activation in the temporal lobe, with lower activation coinciding with greater depression symptoms. The

opposite was true of the superior frontal region where greater activation tended towards greater depression symptoms. Given the tight coupling of activity in these two regions, the over-activation of the superior frontal gyrus may be compensation for the reduced activity in the temporal pole.

Interestingly, the MCI only group was not distinguishable from the asymptomatic group in either task performance or BOLD response to the task. In sporadic MCI, without a history of trauma, performance on the N-back task is generally maintained, though MCI subjects have been found to have increased susceptibility to emotional interference on working memory.[141] Our results in the MCI+MBI group are similar to a previous study of amnestic MCI using a similar fMRI paradigm. In this study, the authors observed that interference on the task was greatest when fearful faces were presented, and that the MCI group showed greater activity in superior frontal regions.[141] The authors also noted increased activation in the precuneus with increasing task demand, as was noted for our MCI+MBI group. Conversely, the authors also noted differences in limbic activation, with the amygdala having greater activation in the MCI group. Contrary to our hypotheses, we did not observe any differences in a between the groups in limbic system activity.

The interaction between attentional control and emotion is thought to be related to two processing streams: the bottom-up emotional stream, involving limbic system regions, and the top-down executive control stream, involving prefrontal and parietal regions. The dissociation of these streams is posited as the mechanism for impairment of working memory in response to emotional interference.[142] It may be the case that for the MCI+MBI subjects, both streams are dysfunctional given the over-recruitment of the precuneus when no distractors were present and the interference of fearful faces on the task. Additionally, the over-activation of the superior frontal regions may reflect an augmented inhibitory response to the distractors. In other words, the MCI+MBI group may be requiring more effort to maintain executive control over the limbic

system. While this extra effort is successful at preventing over-activation of the limbic system, it results in fewer resources available to complete the working memory task successfully, as evidenced by reduced performance in the 2-back condition with fearful faces.

In this study of former professional football players, we observed altered neural recruitment patterns in response to an emotional faces working memory fMRI paradigm in those with both cognitive and behavioral impairments, but not cognitive impairments alone. The behavioral dysfunction of the MCI+MBI group is associated with increased interference of working memory by emotional distractors and altered neural recruitment patterns in response to the task. The expression of behavioral dysfunction in the MCI+MBI group may be related to the inability to maintain executive control in the presence of emotionally-laden distractors.

CHAPTER 5: DISCUSSION

In former professional football players, the effects of recurrent concussion impact both cognition and behavioral function; the risk of developing mild cognitive impairment or depression increases as the number of concussions increases. These effects are likely related to the neurodegenerative disease CTE, which in a recent study was found to be present in at least 9% of former players who died over the study period.[129] While this study was biased towards more severe cases of CTE, the prospect of developing a neurodegenerative disease remains a grave concern for many former NFL players. As these individuals go through the natural aging process, many are concerned that normal changes to their cognition are the result of their exposure to head impacts earlier in life. Being able to identify the early signs of CTE-related cognitive or behavioral decline is an important goal to better counsel these patients and determine their risk of progressing to more advanced impairment, including dementia.

This study sought to address this gap in our understanding of early decline in the highly head impact-exposed population of former professional football players. We identified cases of mild cognitive and behavioral impairment using operational definitions that are consistent with previous research. While such classification systems are always imperfect at distinguishing normal from abnormal, we chose to use instruments that take into account informant report and the effects the impairment on daily living. Informant report has been shown to be a better predictor of future development of dementia.[143,144] It is worth noting, however, that in this population, there is high awareness of a possible relationship between neurodegeneration and concussion exposure. Thus, the informant viewpoint may have greater sensitivity, possibly at a cost of specificity, in recognizing mild impairments.

There are limitations to the current work and exciting future directions in the secondary analyses. A wide and varied mixture of neuropsychiatric symptomatology was expressed to varying levels in our MCI and MBI+MCI groups. Secondary analyses of subpopulations within the MCI and MCI+MBI groups would be interesting (e.g. subjects with irritability and depression, more severely impaired subjects), but are outside the scope of the present work. It would also be important to consider variable neuropsychiatric symptom expression in the analysis of the task-based fmri data, which had high variance in frontolimbic neural activation, particularly in the subcortical regions. By relaxing the multiple comparisons correction set a priori, we may observe greater signal within this region, particularly in the task contrasts of emotional faces. By reducing the number of weak responders to the task from the group, we may detect greater group differences between subjects with specific impairments.

In the first aim of this study, we characterized the domains of cognition and behavioral symptoms that are involved in early decline. We found that in the subjects with impairments, memory and executive function appear to be involved, with corroborating evidence from paper and pencil cognitive assessments, the NIH Cognition Toolbox, and the fMRI working memory paradigm. For subjects with behavioral impairments, aggression and irritability were among the most common symptoms reported, though surveys completed by the subjects suggest large effects on anxiety and depression, but less so for aggression. The informants reported high levels of disinhibition, as well as apathy or indifference.

Our next two aims concerned the underlying process affecting the brain's structure and function. Using diffusion-weighted imaging, we found that both impaired groups had lower white matter integrity in the bilateral uncinate fasciculi, though it is unclear what cellular process is causing this reduction in integrity. Interestingly, the MCI and MCI+MBI group were indistinguishable in terms of white matter integrity, suggesting a common underlying disruption may result in either, or that MBI is not related to structural damage in the frontolimbic network.

Conversely, using task-based fMRI, we found that the MCI+MBI group performed worse on the fMRI task and showed greater alteration in neural recruitment. Here, the MCI+MBI group drove group differences while the MCI only group performed as well as the asymptomatic subjects and had similar neural recruitment patterns.

Concussion has been called both a structural and a functional injury. In this study, we found evidence for structural damage in the subjects with either MCI or MCI+MBI relative to the asymptomatic subjects. We also observed functional disruption in the MCI+MBI group relative to the MCI and asymptomatic subjects. This suggests the cognitive component of impairment is related to structural damage, while behavioral impairments are related to function. However, the methods used in this study cannot fully address this issue. The behavioral impairments may be related to damage outside of the frontolimbic network. In any case, both diffusion-weighted imaging and functional MR are proxy measures for the pathological process we are intending to study. Determining the in vivo burden of tau deposition is a critical step towards understanding the course of CTE.

The primary future direction of this study is the prospective follow-up of these subjects over the next several years, tracking the progression, if any, of their impairments. Additionally, some subjects in the asymptomatic group may develop pathological cognitive or behavioral symptoms. We have previous assessments on 23 of the subjects in this study; these subjects have participated in previous research involving neuropsychological testing and MR imaging. Of these subjects, six have developed MCI or MCI+MBI since their last assessment, which occurred 4-8 years prior. In addition, two of the excluded subjects developed full dementia since they were last seen. By systematically following and reassessing these subjects, we may begin to understand the natural history of CTE, rather than relying on the snapshots in time that much of the research has been thus far been limited to.

Future studies would be improved by including a control group of healthy, unexposed individuals to which the exposed subjects, impaired or not, could be compared. Perhaps more importantly, a clinical comparison group is needed. This group would include subjects that are unexposed, but who have mild cognitive or mild behavioral impairments. By comparing sporadic cases of MCI or MBI to cases in the former professional football cohort, it may be possible to develop distinguishing criteria for CTE-related degeneration. A key component to this future study would be the confirmation of CTE, or at least a strong in vivo marker of CTE (e.g. by using NFT-binding PET tracers).

In summary, this study improved our understanding of the effects of recurrent trauma and the development of mild cognitive and behavioral impairments in subjects with a known and significant exposure to the concussive and subconcussive impacts common to American football. We observed both structural and functional evidence of these impairments, which may reflect an underlying neurodegeneration. With a critical mass of CTE cases identified in this population, continued work is needed to improve our knowledge of the pathological consequences of head impact exposure. Early identification is a critical step towards being able to prognose further decline and counsel patients appropriately. Neuroimaging is a powerful tool that may help identify objective markers of CTE and targets for therapeutic intervention. The ramifications of this research extend well past the relatively small population of former professional football players. The much larger body of collegiate and high school contact sport athletes and military personnel exposed to recurrent concussion will benefit from this research.

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